Development, Characterization and Evaluation of Self Nano Emulsifying Drug Delivery System (SNEDDS) of *Calotropis procera* Linn. for Anti-diabetic Activity

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Ayurvedic Pharmacy

By

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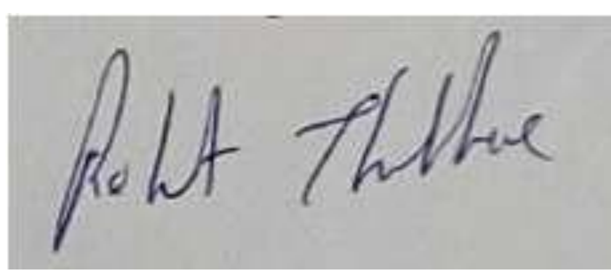
2023

DECLARATION

I hereby declared that the presented work in the thesis entitled "Development, Characterization and Evaluation of Self Nano Emulsifying Drug Delivery System (SNEDDS) of *Calotropis procera* Linn. for Anti-diabetic Activity" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision Dr. Manish Vyas, working as Assistant Professor, in the School of Pharmaceutical Sciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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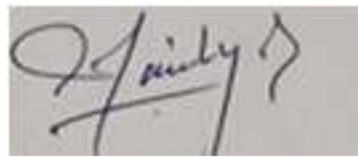
CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Development, Characterization and Evaluation of Self Nano Emulsifying Drug Delivery System (SNEDDS) of *Calotropis procera* Linn. for Anti-diabetic Activity" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the School of Pharmaceutical Sciences, is a research work carried out by Rohit Thakkar, 41700108 (Registration No.), is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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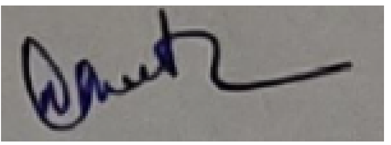

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Abstract

Ethnopharmacological relevance:

Calotropis procera Linn. is reported to have a wide array of therapeutic activities including hepatoprotective, anti-diabetic, antioxidant, antimicrobial, antiviral, antiplasmodial, and diuretic. It has been used since ancient times and reported to have multidimensional therapeutic activities. Plants are widely distributed in tropical and subtropical areas of the world and easily available in agriculture and non-agriculture fields. Traditionally, it is used in the powder form which has drawbacks including high dose, palatability, and stability.

Objective: The present study was designed for the development and characterization of the Self Nano Emulsifying Drug Delivery System of ethanolic extract of *Calotropis procer*a leaves. Optimized batch of prepared SNEDDS were evaluated for its anti-diabetic potential by using *in-vitro* and *in-vivo* studies.

Methods: Twenty-seven formulations of SNEDDS were prepared based on solubility studies where labrafil as oil, tween 80 as surfactant and transcutol as co-surfactant was selected. The optimized batch was characterized by entrapment efficiency, droplet size, polydispersity index (PDI), zeta potential, transmission electron microscopy, Dissolution study and accelerated stability study. For Antioxidant and Anti-diabetic activity, DPPH assay and α-amylase activity was performed. Further, *In-vivo* study was concluded on streptozotocin-induced diabetes in rats.

***Keywords*: *Calotropis procera*, SNEDDS, Anti-oxidant, Anti-diabet**

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Firstly, I would like to thank God almighty for giving me wisdom and knowledge to showcase my talent. I want to express my gratitude and special thanks to my guide Dr. Manish Vyas and Co-guide Dr. Navneet Khurana who for his expelary guidance, monitoring, and constant encouragement throughout the course of this thesis and who despite being extraordinarily busy with his duties, took time to hear, guide and keep me on the correct path. The blessings, help and guidance given by him time to time shall carry me a long way in the journey of life on which I am about to embark. I also express my thanks to my parents, my wife, and my son for their unconditional support with my studies. I am honored to have you as my family for encouraging me in all my pursuits and inspiring me to follow my dreams. I also express my thanks to my brother, for me you always have been a source of motivation and strength during moments of despair and discouragement.

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Chapter-1

1.1 Introduction

Diabetes is one of the major and deadly metabolic disorder. An abnormal metabolic reaction may disrupt the natural process which may cause chemical changes and results in causing the disease. When insulin level disturbed either by its secretion or action or may be both, it causes diabetes and it is characterized by hyperglycaemia. Failure of heart, blood vessels, disinfection in nerves, eyes, different organs especially kidneys and long-term chronic disease is mainly associated with chronic hyperglycemia.[1,2]. Diabetes comes under the top ten causes of deaths worldwide, about 1.5 million deaths are directly reported each year and the rate of death is increasing every year. Diabetes will increase up to 114% from 37% in 2030 according to the research of world population by World health organisation (W.H.O). This is because the world population in 2025 will reached expected to 7.9 billion. In 2003, 5.1% of population in worldwide was diabetic i.e., 194 million of total population and with this 8.2% of world population had impaired glucose tolerance. The prevalence increased to 6.0% and 8.0% in 2007. The global diabetes prevalence in 2019 was estimated to be 463 million, rising to 700 million in 2045[2]. Many conventional medicines are available for the management of diabetes, some of the common medicines are metformin, tripeptide, dapagliflozin, empagliflozin etc. and insulin therapy. However, these managements have also been reported to have various side effects and complications, but these side effects and complications can overcome by using alternative treatments especially herbal drugs because their natural compounds such as proteins, xanthine's, tannins, flavanol, flavonoids, lignans have been examined by different authors for diabetes management. Plants have been utilised for medication from ancient times in traditional system of medicine and well accepted as reliable source for drug assessment and development[2,3]. So, plant-based drugs are being screened for the better alternative of such medicines. Medicinal plants are the essential origin of medicines from ancient times and used in the traditional healthcare system for years ago. 5.86 billion inhabitants depend on traditional medicines, reported by W.H.O. and it is around 80% of the world population.

(Ahsan et al., 2009). However, information on their safety is not adequate available. This is due to fact that herbs are usually safe, simply because they are obtained from natural sources (Afolayan et al., 2009). Currently, plants are not only the source of medicine for the tribe or traditional healers but also used and explored by scientists because plant-based medicines have better safety and efficacy and also are economic. One of such plant is *Calotropis procera* Linn. (*C. procera*), which has been reported to have the anti-diabetic activity including the other therapeutic activities. It is found in most of the agriculture and non-agriculture fields. The advantage of the plant is that it is neither consumed by grazing animals nor require cultivation practices. It is widely distributed in tropical and subtropical area in India and throughout world. *C. procera* belongs to the Asclepiadaceae family and is commonly known as Arka. It has two different species including *Calatropis procera* and *Calatropis gigantia* Linn. Both the varieties were reported to have a similar phytochemical profile. Every part of *C. procera* including root, bark, flowers, leaves, stem has been used for medicinal purposes and reported to have multidimensional pharmacological actions including anticancerous, antidiabetic, and antimicrobial etc.[4] The study carried out by Mohammad and Etuk in 2009 reveals that the water soluble extract of *C. procera* shows significant results in hypoglycaemic activity in alloxan-diabetic rats. But the challenge of herbal extract over other formulation is the solubility, bioavailability, stability and repeated dose administration. Recently nanotechnology being explored for the development of herbal drug industry to address the various drawbacks including the dose, solubility, stability etc. It can deliver the adequate concentration of the active molecule at the desired size due to their unique and small particle size. Hence, this study is planned to develop the Self Nanoemulsifying Drug Delivery System (SNEDDS) with the ethanolic extract of *C. procera* to overcome the drawbacks related to the plant-based medicines.[4,5] The ability to improve new substances such as by increasing selectivity and efficacy reducing side effects, toxicity and controlling the release of active constituent makes this approach even more attractive.[5,6]

The work for *C. procera* has been reported on plant-based formulations which have various challenges associated with their dose, solubility, bioavailability, toxicity, and stability. But the study shows that the plant has good anti-diabetic properties. However,

no data for SNEDDS on *C. Procera* is available. So, the current study is planned to overcome the above-mentioned issues. Review of some previous work is listed below.

Review of previous work:

The work shows the anti-diabetic property of *C. procera* :-

- 2010- Kokil GR, Rewatkar PV, Verma A, et al. Pharmacology and chemistry of diabetes mellitus and antidiabetic drugs: a critical review. Curr Med Chem 2010; 17: 4405 – 4423
- 2013- Jaiswal, J. A. T. I. N., et al. "Anti-diabetic activity of methanolic extract of Calotropis gigantea seeds on STZ induced diabetic rats." *Int. J. Pharm. Pharm. Sci* 6.1 (2013): 254-257.
- 2013- Neto, Mário CL, et al. "Evaluation of antihyperglycemic activity of Calotropis procera leaves extract on streptozotocin-induced diabetes in Wistar rats." *Revista brasileira de farmacognosia* 23.6 (2013): 913-919.
- 2016- GUPTA, AMIT, and SUSHAMA R. CHAPHALKAR. "Anti-diabetic activity of Calotropis gigantea in human whole blood." *HEALTH* 2016.6 (2016): 3
- 2017- Manivannan R, Shopna R, Antidiabetic activity of Calotropis gigantea white flower extracts in alloxan induced diabetic rats, Journal of Drug Delivery and Therapeutics. 2017; 7(3):106-111

Chapter-2

Literature review

2.1 Arka[6,7]

Botanical name: *Calotropis procera* Linn.
Family: Apocynaceae

2.1.2 Ayurvedic Properties

Rasa - Tikta, Katu

Guna - Laghu

Veerya - Ushna

Vipaka - Katu

Karma - Kaphavatashamak, kaphapittashamak

2.1.3 Arka in Samhita:

Samhita	Description	References
Charak	*Kshee rivriksha* and *Shatashodhan Vriksha* Root in *Shirovirchen Dravya*	Cha.sam. 1/116, 5/53
Sushruta	*Kashara Nirmaan* and *Dravya* of *Arkadi Gana*	Su.su 11/11, 14/36
Astang hrudya	*Kaphanask* and *Dantapawana*	A. H. Su. 2/2, 29/76
Kashyap	Used in A*bhyanga* and *Pariseka*	Kasy. Sam. 17/81

2.1.4 Arka in Nighantu[7 8]

Sr.No.	*Nigahantu*	*Varga*
1	*Dhanvantari Nigahantu*	*Karaveeradi*
2	*Shodhala Nigahantu*	*Karaveeradi*
3	*Shadarasa Nigahantu*	*Tiktadravya skanda*
4	*Madanpala Nigahantu*	*Abhyadi*
5	*Kaiyadev Nigahantu*	*Oshadhi*
6	*Bhavaprakash Nigahantu*	*Guduchyadi*
7	*Raj Nigahantu*	*Karaveeradi*
8	*Priya Nigahantu*	*Shatapushpadi*
9	*Adarsh Nigahantu*	*Arkadi*
. 10	*Gunaratnamala*	*Guduchyadi*

2.1.5 Distribution[8,9]:

- The herb is present more or less in India.
- It is found in dry places like Afghanistan, Arabia, Egypt, Iran etc.

2.1.6 Chemical Constituents[10]:

- Calotropin and calotropagenin, uscherin, calotropenin, calotropenyl acetate, multi-flavanol compounds.
- Triterpenoids, calotropursenyl acetate and calofriedelenyl, anorditerpenyl esters, chlorophenyl and bioflavonoids, beta-sitosterol

2.1.7 Identity, Purity and Strength

Foreign Matter	Not > 2%
Total ash	Not > 21%
Acid-insoluble ash	Not > 5%
Alcohol soluble ash	Not < 5%
Water soluble extractive	Not < 24%

2.1.8 Macroscopic characteristics of the *Calotropis procera*:

The table number 2.1 is showing the macroscopic characteristics of the different parts of the *Calotropis procera.*

Table 2.1- Macroscopic characteristics of the different parts of the *Calotropis procera*[11]

Habit	Shrub to small tree, 3-8 m. in height
Root	Simple, corky bark, white tomentose branch, branched, woody at the base.
Leaves	simple, opposite, entire margin, cordate, oblong, obovate 3-1.7 cm in length, subsessile
Flowers	Bisexual, actinomorphic, Bracteate, pedicellate, hypogenous, pedicle l.3-5 cm long
Floral characteristic	multiflowered, dense umbellate, axillary
Calyx	polysepalous, quincuncial aestivation, glabrescent

Corolla	gamopetalous, twisted aestivation
Androecium	gynandrous, coherent, anther dithecous.
Gynoecium	apocarpous, bicarpellary, peltate stigma
Fruit	simple, inflated, obliquely ovoid, fleshy
Seeds	Obovate, flat,7x6 mm, compressed

Image 2.1- *Calotropis procera* leaves

Chief advantages of the plant:

- Plant can grow in different environmental and soil conditions.
- Plant itself is a weed and survive in most of the environmental conditions where nothing else grow
- Cultivation practices is not required.
- Grazing animals does not consume this plant.

2.1.9 Microscopy [12,13]:

Leaf: The transverse section of the leaf when seen under the microscope it shows the lamina of the leaf consist of the single layer of epidermis which is covered with thick striated cuticle. Some of the epidermal cells are also present on the upper and lower surface. In the leaves, the parenchymatous cells are also present that are circular. The intracellular shape is present in ground tissues. Below the upper epidermis, the three layers of closely arranged palisade parenchyma cells are present.

2.1.10 Anti-diabetic and other Pharmacological Activity of *C.procera*:

C. procera belongs to Apocynaceae family, is a wild bush originated from Africa, India and Persia (Gomes et al., 2006; Singhal and Kumar, 2009). In Brazil, it was introduced as an ornamental plant. *C. procera* has biologically active substances such as flavonoids, cardioactive glycosides, triterpenoids, alkaloids, resins, anthocyanins, tannins, saponins and proteolytic enzymes (Shaker et al., 2010). The latex of *C. procera* has been widely studied due to its antihyperglycemic (Roy et al., 2005), anti-inflammatory, gastroprotective (Tour and Talele, 2011), antinociceptive and selective cytotoxic effects (Teixeira et al., 2011). The study carried out by Mohammad and Etuk in 2009 reveals that the water soluble extract of *C.procera* shows significant results in hypoglycaemic activity in alloxan-diabetic rats

Other Related Pharmacological activities have been listed below in the table along with the part used, type of extract and dose.

Table 2.2- Reported Pharmacological Activities[13]

Sr. no.	Pharmacological activity	Part used	Extract	Dose
1.	Anti-inflammatory	Aerial	Aqueous and methanolic	100-250 mg/kg[9]
2.	Antifertility activity	Root	Ethanolic extract	1. 250 mg/kg[10]
3.	Hepatoprotective Activity	Flower	Ethanolic extract	200 mg/kg[10]
4.	Antidiarrheal activity	Aerial	Latex	500 mg/kg[11]
5.	Antidiabetic activity	Aerial	Aqueous	400 mg/kg[12]
6.	Antihyperglycemic activity	Aerial	Aqueous	100-400 mg/kg[12]
7.	Antitumour activity	Root	Ethyl acetate	2. 10 mg/ml
8.	Antipyretic activity	Aerial	Ethanolic extract	3. 500 mg/kg
9.	Gastroprotective activity	Stem bark	Aqueous	200-400 mg/kg

2.2 Self Nano-emulsifying Drug Delivery System (SNEDDS)[14,15]

Isotropic mixture of drug, oil, surfactant and co-surfactant which on mild agitation produce an ultrafine droplet of (o/w) nano emulsion is called SNEDDS. Improvement in oral bioavailability of poorly water-soluble drug observed in SNEDDS. This drug delivery system has a distinctive property, that they can self-emulsify fast in gastric fluids and under mild agitation provided by the motion of G.I.T. they form fine (o/w) nano emulsion.

2.2.1 Types of SNEDDS (nano emulsion)[16,17]:

Table 2.3 -Different types of SNEDDS

S.no.	Types of nano emulsion (SNEEDS)	Detail
1.	Water in oil nano emulsion	Dispersion of droplets of water in continuous phase of oil
2.	Oil in Water nano emulsion	Dispersion of droplets of oil in continuous phase of water
3.	Bi-continuous nano emulsion	Soluble in both oil and water phase

2.2.2 Selection of excipients for SNEDDS[18,19]:

Very few excipients are there which is having good property to develop self-emulsifying system because concentrations of oils, surfactants and co-surfactants are playing the major role for the self-emulsification.

Oil:

Oil plays an important role in the emulsification of SNEDDS. It helps in emulsification and increases the lipophilicity via the intestinal lymphatic system. Oil phase for the preparation of SNEDDS should have the maximum potential of solubilization of the

selected drug. It is responsible for the maximum drug loading in nano emulsion (SNEDDS). Some synthetically or chemical modified oils are used to prepare self-nano emulsion such as fatty acids, fixed oils.

Table 2.4- Various oils used in the preparation of SNEDDS[20,21]

S.No	Class	Example	Commercial names
1	Vitamins	Vitamin E	-
2	Fatty acids	Oleic acid and caprylic acid	Coessential 094
3.	Fixed oils	Castor and soyabean oil	-
4.	MCTs	Triglycerides of caprylic acid	Captex 300,355
5.	Medium-chain mono-diglycerides	Mono and di glycerides of the caprylic acid	Imwitor 742, Capmul MCM, Akoline MCM
6.	PG fatty acid	PG monocaprylate	Sefsol 218

Surfactants:

Surfactants are the molecules or ions that is having the ability to prevent interfacial tension as they are adsorbed at the interface. It enhances the self-emulsification ability of SNEDDS, which further improves the bioavailability of partial adsorbable drug or poorly water-soluble drugs.

Table 2.5- Different surfactants used in the preparation of SNEDDS[21]

S.No.	Class	Example	Market name
1	Polysorbate	POE-20-sorbitan monooleate	Tween20, Crillet
2.	Castrol oil	POE-35- Catrol oil	Etocas35 HV,cremphor EI
3.	Sorbitan esters	Sorbitan monoleate Sorbitan monolaureate	Span 80 Span 20
4.	POE-Vitamin E	POE- Vitamin E	Vitamin E TPGS
5.	POE-PPO	Poloxamer 188	Lutrol F 66
6.	Stearate	PEG-660-12-hydroxysterate	Solutol HS 20

Co- surfactants:

An effective self- emulsify formulation need a high concentration of co-surfactant. It increases the solubility of high amounts of hydrophilic or hydrophobic drugs in the lipid and oil base.

Table 2.6- Co surfactants used in the preparation of SNEDDS

S.No.	**Class**	**Example**
1	Short-chain alcohol	Ethanol
2.	Alkanes, triols	P.G.

3.	Glycol ethers	Diethylene glycol monomethyl ether (Transductal)
4.	Polyethylene glycol	PEG 400
5.	Glycofurol	Tetrahydrofurfuryl alcohol Poly ethylene glycol ether

2.2.3 Characterization of SNEEDS [22]:

SNEEDS are characterised by the number of parameters. These parameters optimize the result. These results may help in the successful commercialization of the product.

Table 2.7- Different parameters of Characterization

S.No.	Parameters	Description
1	Entrapment efficiency	Total drug loading is determining in the formulation
2.	Particle size	Determine the droplet size of the formulation
3.	Polydisperse index	The measure of droplet size homogeneity
4.	Zeta potential	Determine the charge of the oil droplet of SNEEDS
5.	TEM	Transmission electron microscope (TEM) analysis determine the shape of dispersed oil droplets
6	Dissolution Study	Determines the dissolution rate of SNEDDS
7	Accelerated Stability	To analyse the stability of SNEDDS

	Study	

2.2.4 Advantage of SNEDDS[23,24]

2.2.4.1 Stability or entrapment efficiency:

It provides improved physicochemical stability profile over long-term storage and having the advantage of 100% entrapment capacity.

2.2.4.2 Reduction in the dose of drug:

SNEEDS provide improved bioavailability, drug loading capacity and therapeutic efficacy of many hydrophobic drugs. Increase in the bio availability with increase in drug loading capacity lead to the drug dose reduction and its side effects.

2.2.4.3 Easy manufacturing and scale- up

These two are the main factors which utmost used in industries. Method used for preparation, such as mixing with agitation and volumetric filling instrument offers easily manufacturing at big scale with economic benefits.

2.2.4.4 Pharmaceutical formulations:

The number of formulations can be prepared from SNEDDS such as foam, sprays, creams, ointment, gels and it is used as nano emulsion used in the pharmaceutical field such as oral, topical etc.

Chapter-3

Hypothesis

C. procera is a potential herb with many curative principles and economic values. The plant is being used since ancient time in different Ayurvedic scriptures for various therapeutic properties. Recent investigations based on the *C. procera* have also been describing its role in the treatment of chronic diseases like diabetes. Each part of *C. procera* has the therapeutic importance may be due to the presence of a wide range of active chemical constituents. However, plant-based formulations have been reported to have challenges associated with their dose, solubility, bioavailability, toxicity, and stability. Therefore, nano drug delivery systems were explored to address the forementioned drawbacks of such formulations. Besides, it is also able to deliver the adequate concentration of the active molecule at the desired size due to their unique and small particle size. Hence, in the present study, SNEDDS (Self Nanoemulsifying Drug Delivery System) was developed with the ethanolic extract of *C. procera* were to address the drawbacks related to the plant-based medicines.

Chapter-4

Aim and Objectives

Aim: Development, Characterization and Evaluation of Self Nano Emulsifying Drug Delivery System (SNEDDS) of *Calotropis procera* Linn. for Anti-diabetic Activity

Objectives:

- Authentication of the drug *C. procera*
- Optimisation and development of SNEDDS of *C.procera*
- Characterization of the prepared SNEDDS of *C. procera*
- Evaluation of the optimized SNEDDS of *C. procera* for its *in-vivo* antidiabetic activity

Methods

1) Analysis of raw material

A) Organoleptic characteristics

- Colour
- Odour
- Touch

B) Physicochemical analysis

- Determination of foreign matter
- Determination of total - ash
- Determination of acid - insoluble ash
- Determination of alcohol - soluble extractive

C) Qualitative analysis

- Test for flavonoids
- Test for alkaloid
- Test for tannins
- Test for the phenolic compound
- Test for coumarin compounds
- Test for reducing Sugar

- Test for quinones
- Test for proteins
- Test for fixed oil and fats
- Steroidal glycoside
- Anthraquinone glycoside

D) HPTLC

E) Safety Parameters

- Microbial overload
- Pathogen study
- Pesticide study
- Heavy metal analysis
- Aflatoxin study

2) Development of SNEDDS

- Preparation of extract
- UV spectroscopic analysis
- Solubility study
- Preparation of SNEDDS

3) Characterization of SNEDDS

- Entrapment efficiency
- Zeta potential
- Poly dispersity index
- Transmission electron microscopy
- Dissolution study
- Accelerated stability study

4) *In-vitro*

a) Antioxidant study

- 2,2-diphenyl-1-picryl-hydrzyl-hydrate (DPPH Assay)
- Hydrogen peroxide study

b) Antidiabetic study

- α-amylase study

5) ***In-vivo* Antidiabetic study**

- Streptozotocin induced diabetic Method

Chapter-5

Methods and Materials

5.1 Methodology for achievements of the objectives:

5.1.1 Collection of drug: The leaves of the plant collected from the *Ayush Vatika* of Lovely Professional University, Phagwara (Pb.)

5.1.2 Authentication of drug: *C.procera* was authenticated from "National Institute of Pharmaceutical Education and Research" (NIPER), Mohali (Pb.), India.

5.1.3 Organoleptic study: Arka leaves were sensory observed for colour, odour, texture.

5.1.4 Physicochemical Analysis of *C. procera*[25,26]

Following parameters were employed for the physicochemical analysis of the *C. procera:*

5.1.4.1 Foreign matter:

100 g of sample was spread in a stainless-steel tray and foreign matter was sensory observed. Foreign matter was removed and remaining quantity of the sample was weighed and foreign matter percentage is calculated.

$$\text{Foreign matter} = \frac{\text{Weight of foregin matter}}{\text{Weight of drug}} \times 100$$

5.1.4.2 Acid insoluble ash:

25 ml dilute HCL acid was mixed in the ash and boiled for 5 minutes. Ashless filter paper was used for filtration. Hot water was used for the washing of the filtrate and subjected to muffle furnace for the ignition to achieve the constant weight. Percentage of acid insoluble ash was calculated.

$$\text{Acid insoluble ash} = \frac{\text{Weight of residue} \times \text{Volume made}}{\text{Weight of Sample} \times \text{Volume taken}} \times 100$$

5.1.4.3 Alcohol soluble extractive:

100ml of alcohol was added in a closed conical flask with 5gm of the drug. Up to 6 hours the conical flask was shaken and kept undisturbed for 18 hours, then filtered. 25 ml of filtrate was subjected to evaporate in China dish. The alcohol soluble percentage was calculated.

$$\text{Alcohol soluble value} = \frac{\text{Weight of the residue} \times \text{Volume made}}{\text{Weight of the Sample} \times \text{Volume taken}} \times 100$$

5.1.4.4 Water soluble extractive:

100ml of alcohol was added in a closed conical flask with 5gm of the drug. Up to 6 hours the conical flask was shaken and kept undisturbed for 18 hours, then filtered. 25 ml of filtrate was subjected to evaporate in China dish. The water-soluble percentage was calculated.

$$\text{Water soluble value} = \frac{\text{Weight of the residue} \times \text{Volume made}}{\text{Weight of the Sample} \times \text{Volume taken}} \times 100$$

5.1.5 Qualitative analysis of *Calotropis procera*[27]

Following qualitative tests were performed to investigate different Phyto constituents present in the leaves of *C. procera.*

5.1.5.1 Test for flavonoids (Shinoda test):

In methanol 1-2 ml extract was dissolved by heating. To the methanolic solution 3 pieces of magnesium chips were added with few drops of concentrated HCL acid. Flavonoids presence was indicated by pink colour.

5.1.5.2 Test for alkaloids:

- **Mayer's test:**

Turbidity or yellow/white precipitate formation indicates the alkaloids presence when 1ml aqueous extract acidified with few drops of 1M HCL acid and 6-7 drops of potassium mercuric iodide.

- **Dragendroff's test:**

The extract dissolved with HCL acid was filtered and treated with Potassium Bismuth Iodide solution. Alkaloid is present if red precipitates will come.

5.1.5.3 Test for Tannin:

Extract was filtered after boiling in water bath. Adding few drops of ferric chloride changes to blackish-green precipitates confirms the presence of tannins.

5.1.5.4 Test for the phenolic compound:

Four drops of 1% $FeCl_3$ sol. was added into 3 ml of 1% extract. The deep violet colour was not produced which should be produced in the presence of phenolic compounds.

5.1.5.5 Test for coumarin compounds:

2 -3 ml of extract was treated with 4-5 drops of 1% KOH in absolute ethanol. Yellow colour formation confirms the presence of coumarins

5.1.5.6 Test for Reducing Sugar (Fehling's reagent test):

2 ml of plant extract was added in a test tube with 1-2ml of Fehling reagent A and B and mixed, for 15 min. this solution heated on water bath. Reducing sugar is indicated if brick red or orange colour precipitate appears.

5.1.5.7 Test for Quinones:

Sodium hydroxide is added to the sample. Appearance of blue, red or green colour confirms the presence of quinones.

5.1.5.8 Test for Proteins (Xanthoproteic Test):

4-5 drops of concentrated nitric acid was added to the extract, the yellow colour of the solution if appears confirms the presence of proteins the sample.

5.1.5.9 Test for fixed oil and fats:

Two drops of the concentrated extract were applied on filter paper and pressed by other filter paper in between and kept undisturbed. Appearance of oil stains in the filter paper confirms the presence of oils and fats.

5.1.5.10 Steroidal glycoside (L.Buchard test)

Acetic anhydride was used to dissolve the dry extract, heated for boiling, and cooled. To this 1 ml of conc. sulphuric acid was added. Green colour formation indicates the presence of steroidal glycoside

5.1.5.11 Anthraquinone glycoside (Borntragger test)

To the test sample 4ml KOH is added, red colour indicates the presence of Anthraquinone

5.1.6 High Performance Thin Layer Chromatography (HPTLC)[29,30]

5.1.6.1 Procedure

For test solution extract 1gm drug with petroleum ether (60-80°C) (3x15 ml) under reflux on a water bath. Petroleum ether extract was concentrated and volume was made up to 10 ml with petroleum ether. For standard solution 2 mg of alpha –amyrin was dissolved in 10 ml of methanol. Toluene: Ethanol (9:1) was used for solvent system. 10 microlitre of test solutions and 5 microlitre of standard solution was applied to a distance of 8 cm in a precoated silica gel 60 F TLC plate (E. Merck) of uniform thickness of 0.3 millimetre to develop the plates in solvent system.

5.1.6.2 Visualization

For spraying anisaldehyde-sulphuric acid reagent was used, the plate was heated at 70°C until the coloured bands develop, R_f value of coloured band was observed.

5.1.7 Safety Parameters

5.1.7.1 Total microbial plate count[31,32]

Petri dishes 10-12 cm in diameter was used for bacteria. 20 ml of liquified casein-soybean digest agar and pretreated herbal material mixture of 2 ml was added in one dish at a temperature of 45ºC. Material was spread in a petri dish. For colonies count of not more than 300 dilution was done. Material was diluted to get a desired count of colony i.e., not more than 300. Same dilutions of two dishes were prepared inverted them and incubated at 35–45ºC for 2 to 3 days. Formation of total number of colonies were counted and observed the results and calculated.

5.1.7.2 Pathogen test[32,33]:

Pretreated material was homogenised appropriately and incubated at 35–40°C for sufficient growth (4-8 hrs.), but not for multiplication. Container was Shaked. 2ml - 100 ml of Enterobacteriaceae enrichment broth Mossel aliquot homogenized material was transferred and incubated at 40–45ºC for 16–46 hrs. For subculture red bile agar with lactose and glucose was prepared.

5..1.7.2.1 *Escherichia coli:*

Pretreated material was homogenised appropriately and incubated at 35–40°C for sufficient growth (4-8 hrs.), but not for multiplication. Container was Shaked. 2-100 ml of Enterobacteriaceae enrichment broth mussel aliquot homogenized material was transferred and incubated at 40–45°C for 16–46 hrs. red colonies growth indicates the presence of *E. coli.*

5..1.7.2.2 *Staphylococcus aureus:*

Soybean-casein digest medium was inoculated, to the quantity of the solution 1 gm of the suspension material was examined. Resultant was mixed and incubate at 40–45°C for 46-78 hrs. Baird-Parker agar was used for subculture preparation. Incubated at 40–45ºC for 46–78 hrs.

5.1.7.2.3 ***Pseudomonas aeruginosa:***

Soybean casein digest medium was inoculated to the quantity of the solution1 gm of the suspension material was examined. cetrimide agar plate was used for subculture preparation, plate was incubated at 40–45°C for 22–46 hrs.

5..1.7.2.4 ***Salmonella spp.***

Pretreated material was homogenised appropriately and incubated at 35–40°C for enrichment. Enrichment culture of 10ml was transferred to 100ml tetrathionate bile broth and incubated at 35-48°C for 16-28 hrs. Subculture was prepared on at least two agar media: lysine, deoxycholate citrate agar and incubated at 40-45°C for 16-24 hrs.

Table 5.1- Validation of tests for detection of specific micro-organisms

Micro-organism	Strain count	Medium
E. coli	NCIMB 8545 (ATCC 8739, CIP 53)	Lactose medium broth
Pseudomonas aeruginosa	NCIMB 8626 (ATCC 9027, CIP 82)	Soyabean-casein digestive broth
Staphylococcus aureus	NCIMB 8625 (ATCC 6538 P, CIP 53)	Soyabean-casein digestive broth
Salmonella typhimurium	No strain number recommended.	lactose medium broth

5.1.7.3 Aflatoxin Test[34]: Presence of highly toxic contaminants B1, B2, G1 and G2 of aflatoxin can detect by aflatoxin test.

5.1.7.3.1 Stock standard solution: In 50ml of toluene-acetonitrile (9:1) solution 1.0 mg crystalline material of aflatoxins G1, G2, B1 and B2 in glass flask is dissolved and vigorously Shaked to obtain 20 μg/ml standard stock solution Weighed it and dissolved in toluene-acetonitrile (9:1) solution of 60ml. Mixture was then Shaked in a glass flask. Refrigerate the standard solution at 4°C.

5.1.7.3.2 Working standard solution: To obtain 200 ml (50 ng/ml), stock solution of 0.6 ml was added to toluene -acetonitrile (9:1)

5.1.7.3.3 Standard solution: To toluene acetonitrile (9:1) solution, 1.2 ml (2.5 ng/ml) of working standard solution was added.

5.1.7.3.4 Method

Acetonitrile-Ethanol-water (1:3:6) was used as mobile phase. Sonication was used to de-gas the mobile phase. Connected an octadecyl-silica gel column 5.7 mm × 260 mm, 5–7 μm) with a flow rate of 1 ml/ minute, the column was maintained at 50°C. The aflatoxin was observed at 370 nm and 470 nm wavelength. The volume of injection volume used was 25 μl.

5.1.7.4 Heavy metal analysis: It is designed to determine metallic impurities content. Sample of C.P was investigated for the presence of the Lead, Cadmium, Mercury and Arsenic. Inductively Coupled Plasma Optical Emission (ICP-OES) was used for the detection of heavy metals in collected raw materials.

5.1.7.5 Pesticide Residue Test[34]:

For the detection of pesticide residue various methods are adopted. Column and gas chromatography is mostly recommended but can be coupled with mass spectrometry. Chlorine containing pesticide can be detected. Total organic phosphorus measures insecticide contain phosphate. Measurement of total lead and arsenic pesticide was used for the pesticide containing lead and arsenic. Gas chromatography was used for the determination of the pesticide residue content.

5.1.8 Development of SNEDDS

5.1.8.1 Extract Preparation

Fresh and disease-free leaves were selected for the extract preparation. Soxhlet assembly was used for *C.procera* leaves extraction. Leaves were washed and shade dried and then coarse powder was made. Ethanol was used as a solvent for the extraction of leaves of *C.procera* and temperature ranges from 60-65ºC for 24 hrs, so that loss of some heat labile phytochemicals can be protected. The extract obtained was dried on water bath and stored for further experimentation.

Name of Apparatus: Soxhlet
Name of solvent: Ethanol
Temperature:60- 65ºC
Time duration: 24 hrs

5.1.8.2 U. V spectroscopy analysis:

For the linearity of serial dilution of the extract U.V spectrometric analysis study was performed. For UV analysis desired concentration of the extract was taken and calculated. To observe the Linear regression equation absorbance at different concentrations were measured.

5.1.8.3 Solubility Study[35,36]:

Solubility of the extract were evaluated in different types of oils, surfactants and co-surfactants for the preparation of SNEDDS. The pre-determined quantity of the extract was used to mix with oil, surfactant and co-surfactant and stirred well by using digital magnetic stirrer at 150 rpm. At 3000 rpm resulting mixture was centrifuged for 25 min. Supernatant collected and analysed under UV spectrophotometer at 269 nm. Solubility test were repeated in triplicate.

5.1.8.4 SNEDDS Preparation[36,37]

On the basis of the solubility of extract oil, surfactant and co-surfactant 27 batches were formulated. 150 microgram of extract was loaded to each prepared batch for further

sonication by using ultrasonicate for 45 min. 100 ml of distilled water was mixed and stirred in magnetic stirrer (100 rpm) for at 38^0C. Prepared emulsion was visually evaluated for turbidity; it may be milky and opaque also. The transparent emulsion from these 27 batches will be considered as proper nano emulsion.

5.1.9 Characterization of SNEDDS

5.1.9.1 Entrapment efficiency[38]:

10-15 ml of developed SNEDDS was centrifuged at the rpm of 15000 for 3 hrs and supernatant was collected carefully at 24^0C. The Unentrapped prepared nano emulsion was determined spectrometric ally at 269 nm. after dilution.

5.1.9.2 Droplet size and zeta potential[39,40]:

Malvern nano zetasizer (dts ver.5.10) was used to measure the mean of droplet size including polydispersity index of an optimised batch of prepared SNEDDS. These parameters were used to know the size distribution of micro-emulsion. The study was repeated in triplicate.

5.1.9.3 Transmission electron microscopy (TEM)[41]

Under 200000x resolution lens droplet size of the prepared SNEDDS was measured by using TEM (h-600, Hitachi, Japan). 100 ml of water was added to SNEDDS for dilution. It was then evaluated by keeping it on the copper grids having mesh size 400 with films.

5.1.9.4 Dissolution Study[42]: The *in-vitro* dissolution behaviour of SNEDDS of C.P and the extract of C.P was examined with dissolution apparatus. The SNEDDS containing extract of C.P were added into HPMC capsule size 0 then was put into a sinker loaded with 900 ml of stimulated gastric fuel enzymes with pH 1.3, phosphate buffer having pH 6.8 and distilled water at 37°C with a paddle speed of 120 r.p.m

- **Dissolution Apparatus:** USP Type II
- **Dissolution media**: Phosphate buffer with pH 6.8 (900 ml)
- **Temperature:** 37 ± 0.5°C

Process: Type II dissolution apparatus of USP was used to perform in-vitro drug release of drug loaded SNEDDS. Phosphate buffer (pH 6.8, 900ml) was used as dissolution media, rpm was 50 and temperature 40 ± 0.5°C. After suitable time intervals (5, 10, 15, 30 45, 60 and 90 min) aliquots of 5 ml was withdrawn, it was then filtered, diluted and analyzed 269 nm spectrophotometrically under the UV spectrophotometer. All measurements were done in triplicate. *In-vitro* dissolution investigation of the optimized batch of SNEDDS formulation was performed for comparatively study with extract batch.

5.1.9.5 Accelerated Stability study:

5.1.9.5.1 Preparation of Test solution: Sample was evaporated and residue was dissolved in 1 ml methanol. Collected solution was then filtered with 0.45 μm membrane filter. Obtained test solution was then used for HPTLC fingerprinting.

5.1.9.5.2 Preparation of spray reagent: 10 ml glacial acetic acid was mixed with 0.5 ml anisaldehyde, 85 ml methanol and 5 ml of sulphuric acid (98 %).

For Chromatography Toluene: Methyl acetate and methanol in the ratio 8:2:0.5 v/v was used. Chamber saturation time set for 30 min. and test solution was visualised at 254nm, 366nm, 540nm absorbance.

5.1.10 *In-vitro* study[43,44]:

5.1.10.1 Anti-oxidant study

1) 2,2-Diphenyl-1-Picryl-Hydrzyl-Hydrate (DPPH) Assay[44]:

This assay is the most commonly screening tool for antioxidant activity in the compound which are natural. DPPH never degenerates in alcohol and water. Briefly 1 ml of various concentration of different samples were mixed with 1.5 ml of 0.5 mM/L solution of dpph. Mixture was shaked and incubated in dark for 30 minutes absorbance was measured at 518 nm against a blank reagent, for standard ascorbic acid was used. The percentage of DPPH scavenging ability of sample was estimated according to:

$$\text{Radical scavening activity} = \frac{A_i - A_t}{A_i} \times 100$$

A_i - absorbance of control

A_t - absorbance of test

2) Hydrogen peroxide (H_2O_2) scavenging assay[45,46]:

Hydroxyl radical scavenging ability was performed by method as expressed by Ruch et al. With 1 ml of different concentrations of sample, 0.8 ml of H_2O_2 (43mM prepared in 0.1M phosphate buffer) was mixed and incubate for 20 minutes. At 230nm absorbance was checked against reagent blank. hydrogen peroxide scavenging activity of standard and sample was assessed.

$$\text{Radical scavening activity } = \frac{A_i - A_t}{A_i} \times 100$$

5.1.10.2 Anti-Diabetic study

α-amylase inhibition assay[47,48]:

***α-amylase as**say* was performed to access and compared the anti-diabetic activity of extract of *C.procera* and standard. For the determination of alpha amylase activity Starch DNS method was implemented. 100 ml of (0.2M) phosphate buffer was used for the mixing of 500 mg of alpha amylase. To the different concentration of the extract 500 microlitre of alpha amylase was added and incubated at 25°C for 10 min. Then to this 500 microlitre of 1% starch solution added and incubated again for 15 min. at 25°C.Then 1ml of DNS was mixed and boiled by using water bath for 10 min.10ml distilled water was added when it becomes cool and absorbance recorded at 540nm.

5.1.11 *In-vivo* Study[49,50]

5.1.11.1 Animals

Sprague-Dawley Rats of either sex with weight 200 gm-300 gm each were purchased from NIPER, Mohali (Pb.) (CPCSEA registered breeding facility). To avoid transport stress institutional van was used for transportation of animals by road. Polypropylene cages were provided of sufficient size so that animals would freely move in the

cages and also these cages provide the protection from any type of injuries during travelling. Food and water were stored in sufficient containers and in the correct manner to ensure enough food and water during transportation. Central animal house facility at Lovely Professional University (954/PO/ReRcBiBt/S/06/CPCSEA), Phagwara (Pb.) was used to kept the procured animals. 12/12 hr. light and dark cycle were given to animals. Proper humidity conditions and ambient temperature was given to save the animals from environmental effect. Animal experimentation protocol was approved by IAEC of Lovely Professional University with registration no. LPU/IAEC/2021/84

5.1.11.2 Method

High fat diet (HFD) feeding was given to develop insulin resistance, type 2 diabetes in female Sprague-Dawley rats and low dose of Streptozotocin was administered. For the initial period of two weeks rats were divided in to two dietary regimens either high fat diet and normal pellet diet. The preparation of high fat diet includes protein, carbohydrates, fats and multivitamins. STZ was administered in low dose (35 mg/kg, IP) to HFD feeding group after two weeks. After one week of STZ injection, animals were be weighed and the Normal pellet diet and high fat diet rats was subdivided into NPD, NPD + *Calotropis procera*, NPD + SNEDDS, HFD + STZ (-ve control group), HFD + STZ + Acarbose (+ve control group), HFD + STZ + *Calotropis procera,* HFD + STZ + SNEDDS of *Calotropis procera*. The *Calotropis procera* groups were fed orally with 200 mg/kg or 400 mg/kg body weight. The rats were fed as per their respective diets and treated till the end of the study (7 days). P.G.L and P.T.C levels were evaluated on 22nd and 29th day of protocol. Body weight of the animals were checked on day 1st, 15th, 22nd and 29th of protocol. Animals were sacrificed on the 29th day; pancreas of each sacrificed animal was isolated and preserved for biochemical (GSH, CAT, and TBARS) studies.

Table 5.2- The details of the various animal groups required are as given below:

Groups	Name of group	Dose and route of administration	Numbers of Rats
1	Vehicle control	Normal pellet diet + 0.5% carboxy methyl cellulose (CMC) (PO)	6
2	*Calotropis procera* extract *per se*	Normal pellet diet + 400 mg/kg of *Calotropis procera* (PO)	6
3	SNEDDS *per se*	Normal pellet diet + SNEDDS *per se* (PO)	6
4	-ve control	High fat diet + Streptozotocin (IP)- 35 mg/kg	7
5	+ve control	High fat diet + Streptozotocin (IP)- 35 mg/kg + Acarbose (PO)- 10 mg/kg	7
6	*Calotropis procera* extract (low dose)	High fat diet + Streptozotocin (IP)- 35 mg/kg + *Calotropis procera* (PO)- 200 mg/kg	7
7	*Calotropis procera* extract (high dose)	High fat diet + Streptozotocin (IP)- 35 mg/kg + *Calotropis procera* (PO)- 400 mg/kg	7
8	*Calotropis procera* extract – SNEDDS	High fat diet + Streptozotocin (IP)- 35 mg/kg + SNEDDS of *Calotropis procera* (PO)- 100 mg/kg	7
9	*Calotropis procera* extract – SNEDDS	High fat diet + Streptozotocin (IP)- 35 mg/kg + SNEDDS of *Calotropis procera* (PO)- 200 mg/kg	7

(-ve= negative, +ve= positive)

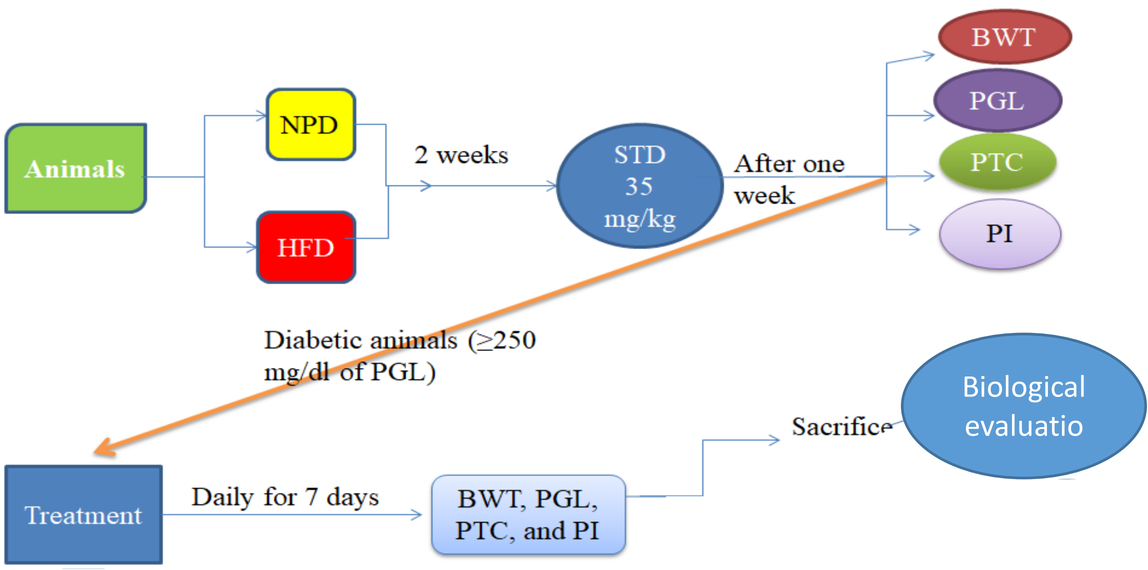


Image no. 5.1 In-vivo study chart

5.1.12 High Fat Diet (HFD)[50]

All the components mentioned in table given below were weighted and then powdered except desi ghee. Required amount of desi ghee was melted and used to triturate the powdered mixture. Small balls were made, they were stored at -20°C and cool to room temperature before giving it to the animals.

Table 5.3- HFD components

S. No.	Components	Weight (g per kg)
1	Normal pellet	365
2	Desi ghee	310

3	Casein	250
4	Cholesterol	10
5	Mixture of vitamins and minerals	60
1.	L-methionine	3
2.	Yeast powder	1
3.	Sodium Chloride	1

5.1.13 Collection of blood samples[50]

Blood was collected from the tail vein during the protocol under light ether anesthesia; the animal was contained and xylene was used to clean the tail and the vein was visualized, a 26 G needle was used to pierce the vein and blood was collected through a 1 ml syringe. The blood was quickly transferred to a 2 ml Eppendorf tube containing 20 µl of saturated EDTA solution. The tubes were run at centrifuge 10,000 rpm for 10 minutes. Plasma (pale yellow supernatant) was used for the estimation of PGL and PTC

5.1.14 Estimation of plasma glucose level[51]

5.1.14.1 Principle

PGL was evaluated using a commercially available kit from Erba Diagnostics Mannheim GmbH Mallaustr, Germany. The principle of the method is based on amount of glucose present in the sample to be oxidized by the glucose-oxidase enzyme to generate gluconic acid and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide further reacts with phenol and 4-aminoantipyrine to give a red dye of chinoneimmina complex. The absorbance of the produced color is directly proportional to the glucose quantity present in the sample when the absorbance is measured at 505 nm and the PGL is expressed in milligrams per deciliter.

5.1.14.2 Procedure

Ten micro liters of supernatant (plasma) were mixed with 1 ml reagent in a test tube, 10 µL of DW with 1 ml of white reagent and 10 µL of standard solution with 1 ml of reagent. At 37°C tubes were incubated for 15 minutes and 4000 µL of DW were added to all the tubes; Absorbance was recorded at 505 nm. The amount of plasma glucose was calculated by the formula:

Glucose (mg / dl) = $\frac{AT}{AS}$ x 100

AT- absorbance of the test samples

AS - absorbance of the standard

5.1.15 Estimation of total cholesterol level (PTC)[51]

5.1.15.1 Principle

The total plasma cholesterol level was calculated using the commercially available kit from Erba Diagnostics Mannheim GmbH Mallaustr, Germany. The method is develop on the principle that the cholesterol esters present in the sample is hydrolyzed from the cholesterol esterase to release cholesterol. Released cholesterol is oxidized by cholesterol oxidase and produce hydrogen peroxide which further reacts with 4 - aminoantipyrin and phenol in the presence of peroxidase to produce a quinonimine red complex. Intensity of colorize is directly proportional to the amount of glucose present in the sample when absorbance is measured at 505 nm. The blood glucose level is expressed in milligrams per deciliter.

5.1.15.2 Procedure

Twenty microliters of supernatant were mixed with 1 ml of reagent in a test tube, 20 µL of distilled water with 1 ml of white reagent, 20 µL of standard solution with 1 ml of standard reagent. The tubes were incubated at 37^0C for 10 min. and 4000 µl of distilled water was added to all the tubes. At 505 nm absorbance was recorded and serum cholesterol was calculated as;

$$\text{Cholesterol in mg / dl} = \frac{AT}{AS} \times 200$$

where AT - absorbance of the test samples and AS - absorbance of the standard.

5.1.16 Homogenate preparation[52]

The pancreas isolated from all rats was homogenized in 0.2 M phosphate buffer at a concentration of 10% (w/v), the homogenate was centrifuged for 5 minutes at 4^0C and 10,000 rpm. The supernatant was utilized for the evaluation of GSH, TBARS, and CAT.

5.1.16.1 Estimation of Glutathione-S-Transferase (GSH)

GSH content in tissue homogenate was estimated as per the method of Beutler *et al.*, 1963. Trichloroacetic acid (10% w/v) in equal volume was used to mix the supernatant of the pancreas homogenate, tubes were centrifuged for 10 min at 4^oC and 10000 rpm. Supernatant was collected and Na_2HPO_4 (0.3 M; 2 mL) was mixed in 0.5 ml of the supernatant with and DTNB 0.001M in 1% w/v sodium citrate (0.25 ml) was added to it. Absorbance of the samples were recorded at 412 nm using UV spectrophotometer.

5.1.16.2 Estimation of Thiobarbituric Acid Reactive Substances

TBARS estimation was conducted as per method of (Ohkawa et al., 1979). Supernatant (200µL) was added in to a test tube, then 200µL SDS (8.1%), 1500µL acetic acid (30%; pH 3.5), 1500µL TBARS was added. 4000µL of distilled water was used for volume adjustment. The test tubes were incubated for 1 hr. at 95^0C, then cooled and 1000µL distilled water was added with 5ml *n*-butanol-pyridine mixture. Tubes were centrifuged for 10 min at 1000 rpm. Absorbance was measured at 535 nm and calibration curve was prepared using 1-10 NM of 3-tetra methoxy propane. The results were expressed as nano- moles per/ mg of protein.

5.1.16.3 Estimation of catalase (CAT)

50 µl of supernatant was transferred in to a test tube containing 1950 µl of phosphate buffer (50 mM; pH 7.0), Changes in absorbance was recorded at 0 sec, 15-sec, and 30 sec at 240 nm after the addition of1000 µl of hydrogen peroxide (30 mM). The CAT was calculated using the millimolar extinction co-efficient of hydrogen peroxide,

activity was expressed in micromoles of hydrogen peroxide oxidized per min. per mg of protein.

$$\text{CAT activity} = \frac{\delta\ \text{O. D.}}{\varepsilon\ \text{X Vol. of sample (mL) X mg of protein}}$$

'δ O.D.' - changes in absorbance/minute

'ε' - extinction co-efficient of hydrogen peroxide

5.1.16.4 Statistical analysis

Sigma Stat 4.0 v. software was used for the statistical analysis. The results are represented as the mean ± S.E.M. For the analysis of variance test, One-way analysis of variance test was used (ANOVA); followed by Tukey test. Results were considered statistically significant at $P<0.05$, $P<0.01$ and $P<0.001$ values.

Chapter-6

Result and Discussion

6.1 Collection of drug

The source of collection of drug is first and very important step for further evaluation, so it should be collected from the place where it is properly maintained and free from pollution and contaminations. Authentication is the prerequisite step for herbal drugs. The source of collection for the drug is AYUSH *Vatika*, Lovely Professional University, Phagwara, because *Vatika* is maintained according to the norms mentioned by Ayush Department.

6.2 Authentication of drug

The leaves of *C.procera* after collection were authenticated from "The National Institute of Pharmaceutical Education And Research" (NIPER) , Mohali. (Pb.)

6.3 Organoleptic characters

It refers evaluation of drug on the basis of colour, odour, touch. The organoleptic characters of the raw drug were studied according to A.P.I and observations were mentioned below in table 6.1.

Table 6.1- Organoleptic features of *C. procera* leaves

S. No.	Parameters	Observation
1	Colour	Green
2	Odour	Characteristic
3	Touch	Soft

6.4 Physico-chemical analysis[53]: Physicochemical parameter of the drug was evaluated because it impacts the physical and biological activity of the drug, As drug may be incorporated with contaminations like sand, silica and debris etc. may degrade the performance characteristics of the component and if the values mentioned for the drug comes under prescribed limits it means drug clears the physicochemical properties otherwise the drug is adulterated which impacts the properties of drug and fluctuate the results in further studies. Physicochemical studies are a part of proximate analysis for quality attribute. Results of physicochemical parameters were performed according to the procedure mentioned by A.P.I and the results lying under the prescribed limits in the monograph of *C. procera* as shown in table 6.2. Total six batches were made and the results were observed and standard deviation the result shown in table 6.2.

Table 6.2 –Physico-chemical analysis of *C. Procera*

S. No.	Parameters	B1	B2	B3	B4	B5	B6	± SD	A.P.I Limits
1	**Foreign matter %**	0	0	0	0	0	0	0	N.M.T 2%
2	**Total ash %**	12.3	12.8	12.6	12.1	12.7	12.5	12.53±0.02	N.M.T 21%
3	**Acid insoluble ash%**	2.8	3.0	2.7	3.1	2.7	2.9	2.86±0.05	N.M.T 5%
4	**Alcohol soluble extractive value%**	7	6	8	6	7	8	7.0±0.78	N.L.T 5%

5.	**Water soluble extractive value%**	25	25	26	28	24	24	25.3±0.71	N.L.T 24%

6.5 Phytochemical analysis[54]: These parameters were performed to check the quality of the sample as it contains all the phytoconstituents. Different chemical was analysed for the detection of the phytoconstituents with its colour change and precipitation reaction. Leaves of *C. procera* contains number of pharmaceutical important phytochemicals like alkaloids, Tannins, flavonoids etc., whereas anthraquinone glycosides, saponins, fixed oils, fats and reducing sugars were observed absent. The result is shown in table 6.3.

Table 6.3 –Phyto-Chemical analysis of *C. Procera*

Test	Chemical	Result
Alkaloids	Mayer's test Dragendroff's test	Present
Tannins	Ferric chloride test	Present
Flavonoids	Shinoda test	Present
Steroidal glycoside	L. Burchard's test	Present
Anthraquinone glycoside	Bontrager test	Absent
Quinones	Sodium hydroxide test	Present
Proteins	Xanthoprotein test	Present
Fixed oil and Fats	Spot test	Absent

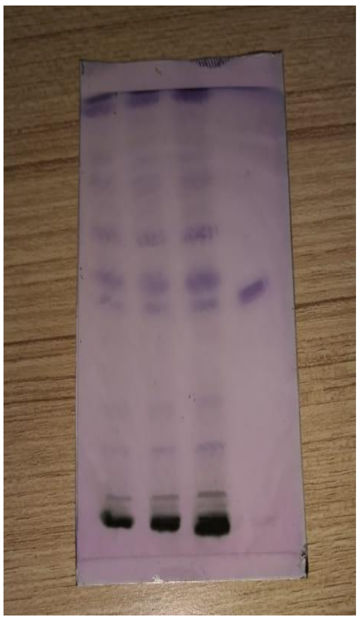
Reducing sugars	Fehling's test	Absent
Coumarins	KOH	Present

6.6 HPTLC Study[55]

HPTLC study performed with the extract taken from Soxhlet extraction method. The HPTLC performed fingerprint confirms the plant identity. For quantitative analytical estimation hyperspectral data, densitograms or image profiles is required. It is an analytical technique evolving sophisticated instrumentation, standardized and documented processors, as well as validated methods. Same sample and plates were used for multiple detection is the strength of HPTLC. Marker compound is used for the purposes of botanical identification, detection of adulteration and as indicator for product quality. Marker compound is a chemically defined constituent of an herbal medicinal product to examine the quality of the sample and not for any therapeutic purpose. HPTLC study was performed and Rf value of extract and standard Rf value was compared. Beta–sitosterol was used as a marker compound as it has been reported to be present in the leaves of *C.procera*. Same Rf value i.e.,0.45 found in the test and standard reveals the quality of the sample. Result is under the following table 6.4

Table 6.4- R_f value of C.P extract and standard of C.P

Sample	**R_f value (Test)**	**R_f value (Standard)**
C.P extract	0.01,0.05,0.07,0.11,0.14,0.21,0.24, 0.30,0.39,0.44,0.50,0.60,0.64,0.69	0.45



T.L.C. Plate

Image 6.1 -Different concentration of C.P and Marker compound

6.7 Safety Parameters

6.7.1 Microbial load[56]: The microbial load test ensures us that how many and which viable microorganisms are present, if microbial contaminations in the drug does not meet the prescribed limit, the drug is being rejected for further evaluation and standardisation as it may be harmful to use because of the presence of microbes. The microbial load is the quantitative determination of microbes. The parameters observed in microbial test were compared with the official monograph for its microbiological quality.

Total microbial plate count and total yeast and mould test study was done and the results come in the prescribed range which estates that sample complies with established standard of microbiological quality. Total microbial plate count was 40 cfu / g, total yeast and mould count was 20 cfu/g which comes under the prescribed limits as described by the monograph. C.P leaves extract comes in the prescribed range given by the official monographs. Result is shown under table no.6.5

Table 6.5- Total microbial count

Sr. No.	Test	Result	Specific requirement
1	Total microbial plate count	40 cfu/g	1 x 105 cfu/g
2	Total yeast and mould	20 cfu/g	1 x 103 cfu/g

6.7.2 Pathogens Test[57]: Pathogen test study was performed to confirm the presence of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella* in the sample. The sample should be free from these harmful chemicals as these degrade the quality and destabilise the drug and its constituents. Pathogen causes disease to the plant so the pathogen test was used as the main criteria to investigate any disease in the plant. Agar well diffusion method was used gram positive (*Staphylococcus aureus* and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). No growth of pathogens was observed in the leaves of C.P extract. Result is shown under table 6.6

Table 6.6- Pathogen test result

Sr. No.	Test	Result	Specific Requirement
1	*E. coli*	N. D	N. A
2	*Staphylococcus aureus*	N. D	N. A
3	*Pseudomonas aeruginosa*	N. D	N. A
4	*Salmonella*	N. D	N. A

(N.D – Not Detected, N.A. – Not Applicable)

6.7.3 Aflatoxin Test: These are natural substances which are produced by moulds and fungi having toxic effects towards human in small concentrations. The aflatoxin producing fungi are widely spread in nature which can contaminate the plants, mainly aflatoxin B1 and B2 and Aflatoxin G1, G2 is harmful and toxic. This test was performed

to check the content of aflatoxin in the drug. There was no aflatoxin found absent in the drug. Result is under table 6.7

Table 6.7- Results of the aflatoxins

Test	Result	Specific requirement
B1,B2 and G1 G2	ND	B1 and G1-NMT 0.5 ppm B2 AND G2-NMT 0.1 ppm

(ND – Not Detected)

6.7.4 Heavy Metal Test[58]: Drug contaminated through heavy metals may lead to various chronic health diseases. U.S. environmental protection agency and international agency for research on Cancer classified these as human carcinogens. Therefore, the plant material used for the preparation of formulation was analysed for the presence of heavy metals within the prescribed limit. Inductively Coupled Plasma Optical Emission-(ICP-OES) technique was implemented for the detection of heavy metal analysis. The parameters observed in heavy metal test were compared with the official monograph. C.P extract was found free from the heavy metals and the range comes under the prescribed limit.

Table 6.8- Results of the heavy metal analysis

Sr No.	Test	Result	Specific requirement
1	Lead (Pb)	ND	NMT 10.3 PPM
2	Cadmium (Cd)	ND	NMT 0.3 PPM
3	Mercury (Hg)	ND	NMT 1.0 PPM
4	Arsenic (As)	ND	NMT 3.0 PPM

(ND- Not detected), (NMT- Not more than)

6.7.5 Pesticide Residue[59,60]: Chromatography was used for the determination of pesticide residues. The raw material evaluated for the presence of the pesticide residues

as recommended by the different official empedium in order to ensure the safety of the raw materials. Hence, the sample was subjected to the detection of the 21 different pesticides in the raw materials and all of them found absent in the sample of *C. procera* extract. Result is shown in the following table 6.9.

Table 6.9- Results of the Pesticide Residue

Sr. No.	Test	Result
1	Pesticide Residue(mg/Kg)	ND
2	Endosulfan	ND
3	2-4’-DDT	ND
4	Permethrin	ND
5	4-4’ DDT	ND
6	Gamma HCH	ND
7	Beta HCH	ND
8	Chlorpyrifos	ND
9	Heptachlor	ND
10	Aldrin	ND
11	4-4’ DDT	ND
12	Dicholevos	ND
13	Malathion	ND
14	Parthion ethyl	ND
15	Parthion methyl	ND
16	2-4’ DDE	ND
17	2-4’ DDD	ND
18	Alpha HCH	ND
19	Dieldrin	ND

20	Deltamethrin	ND
21	4-4’ DDE	ND

ND: Not Detected

6.8 Development of SNEDDS

6.8.1 Preparation of extract[61,62]:

Soxhlet assembly was installed for the extraction of the leaves of C.P. as it allows the isolation of the material even if it has limited solubility in the solvent. Soxhlet extraction ensures that all the analytes are extracted from the plant matrix. It plays vital role to find out actual amount since the raw material becomes continuously heated at the boiling point of solvent for longer period of time which gives highest extraction. Ethanol was used as a solvent for the extraction of leaves of C.P. as it has low toxicity and non-polar capabilities. Ethanol structure enables to dissolves the compounds such as, non-polar and hydrophilic and hydrophobic. The result is shown in table 6.10.

Table 6.10 - Extraction of *Calotropis procera* leaves

S. No.	Wt. of leaves (gm)	Solvent used	Temp (°C)	Duration (Hrs)	Wt. of extract obtained (gm)	Standard deviation
1	25	Ethanol	65	24	1.32	1.40±0.05
2	25	Ethanol	65	24	1.37	
3	25	Ethanol	65	24	1.45	
4	25	Ethanol	65	24	1.44	
5	25	Ethanol	65	24	1.39	
6	25	Ethanol	65	24	1.40	

6.8.2 U.V. Analysis of sample: It is analytical technique for the measurement of the discrete wavelengths of U.V. light which is absorbed by or transmitted through a sample in comparison to a reference sample. U.V. is the overlay spectra of various concentrations. The spectroscopic study was evaluated by using 10mg/ml concentration of the extract. Standard graph was plotted to observe linear regression equation after performing UV analysis in different concentration (1-5 mg/ml). For the determination of the linearity of a serial dilution of the C.P extract U.V analysis was performed. The calibration curve of ethanolic extract of C.P is plotted in figure 6.1 and U.V. absorption of different concentration of C.P extract with standard deviation is shown in table 6.11

Table 6.11 -U.V. absorption of different concentration of *C. procera*

Sr.No.	Conc.(mg/ml)	Absorption			Standard deviation
1	0	0	0	0	0
2	1	0.123	0.131	0.114	0.123 ±0.009
3	2	0.276	0.285	0.266	0.276±0.010
4	3	0.519	0.512	0.525	0.519 ±0.007
5	4	0.658	0.67	0.645	0.658 ±0.013
6	5	0.855	0.842	0.868	0.855 ±0.013

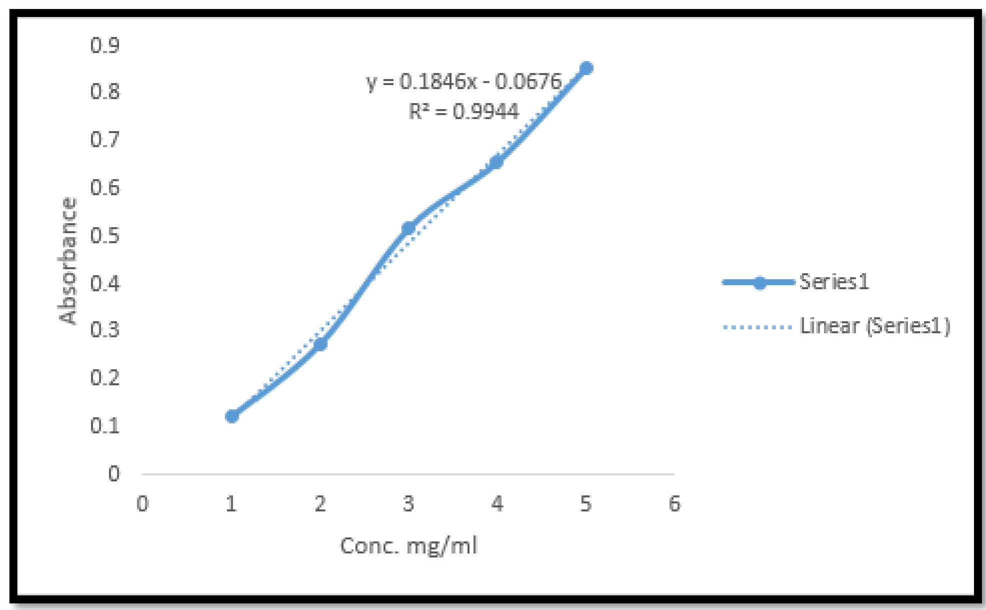


Figure 6.1 : Calibration curve of ethanolic extract of *C. procerra*

6.8.3 Solubility study[63,64]: Solubility is one of the most important physicochemical properties studied during pre-formulation. Solubility studies were performed to investigate the good and most solubilizing capacity of oil, surfactant and co-surfactant for the leaves of C.P extract, labrafil as oil has shown the highest rate of solubility, tween 80 has highest solubilising capacity as surfactant and for co-surfactant transcutol P has highest solubility rate for C.P extract. The solubility study parameters were performed in triplicate. Results of solubility studies were shown in table 6.12 below.

Table 6.12- Solubility of extract of *C. procerra* in various vehicles

S.no	**Vehicle**	**Solubility(mg/ml)**
1	Labrafil	14.124 ±0.88
2	Tween 80	8.92±0.89
3	Transcutol p	7.792±0.92

6.8.4 SNEDDS preparation [65,66]

SNEDDS are anhydrous homogenous mixtures, composed of oil, surfactant, drug and co surfactant which spontaneously form transparent nano emulsion (20-200 nm droplet size) upon aqueous dilution with mild agitation. 27 formulations of SNEDDS were prepared on the basis of solubility studies where labrafil as oil, tween 80 as surfactant and transcutol as co-surfactant was selected, almost 24 formulations were poorly water soluble, which is commonly associated with low bioavailability and lack of dose suitability. Firstly *C. procera* extract was dissolved with Co-surfactant, with gentle stirring for 20 minutes oil and surfactant were mixed and vortexed for some time till the microemulsion prepared and observed for creaming, cracking, turbid and clear nano emulsion. Batch B19 was found clear among all the batches. B-10 batch was also found clear and transparent but it was unstable. Turbidity was observed in B-7, B-8, B-11, B-12, B-13, B-14, B-18, B-21, B-22, B-24. Milky emulsion was served in B-1, B-2, B-20. Creaming was observed in B-5, B-15, B-16, B-17, B-26, B-27. Opaque emulsion was observed in B-3, B-4, B-6, B-9, B-23. Observation of SNEDDS were shown in the following table 6.13, the ternary phase diagram result is shown in figure 6.2.

Table 6.13 - Observation of SNEDDS with various ratios of oil (µl), surfactant (µl) and co-surfactant (µl):

Batch no.	Oil	Surfactant	Co-surfactant	sample	water (ml)	Result
B1	0.1	0.45	0.45	150	100	Milky
B2	0.2	0.40	0.40	150	100	Milky
B3	0.3	0.35	0.35	150	100	Opaque

B4	0.4	0.30	0.30	150	100	Opaque
B5	0.5	0.25	0.25	150	100	Creaming
B6	0.6	0.2	0.2	150	100	Opaque
B7	0.7	0.15	0.15	150	100	Turbid
B8	0.8	0.10	0.10	150	100	Turbid
B9	0.9	0.05	0.05	150	100	Opaque
B10	0.1	0.3	0.6	150	100	Clear
B11	0.2	0.27	0.53	150	100	Turbid
B12	0.3	0.23	0.47	150	100	Turbid
B13	0.4	0.2	0.40	150	100	Turbid
B14	0.5	0.17	0.33	150	100	Turbid
B15	0.6	0.13	0.27	150	100	Creaming
B16	0.7	0.1	0.2	150	100	Creaming
B17	0.8	0.07	0.13	150	100	Creaming
B18	0.9	0.03	0.07	150	100	Turbid
B19	0.1	0.6	0.3	150	100	Clear
B20	0.2	0.53	0.27	150	100	Milky
B21	0.3	0.47	0.23	150	100	Turbid
B22	0.4	0.40	0.2	150	100	Turbid
B23	0.5	0.33	0.17	150	100	Opaque

B24	0.6	0.27	0.13	150	100	Turbid
B25	0.7	0.2	0.1	150	100	Turbid
B26	0.8	0.1	0.07	150	100	Creaming
B27	0.9	0.07	0.03	150	100	Creaming

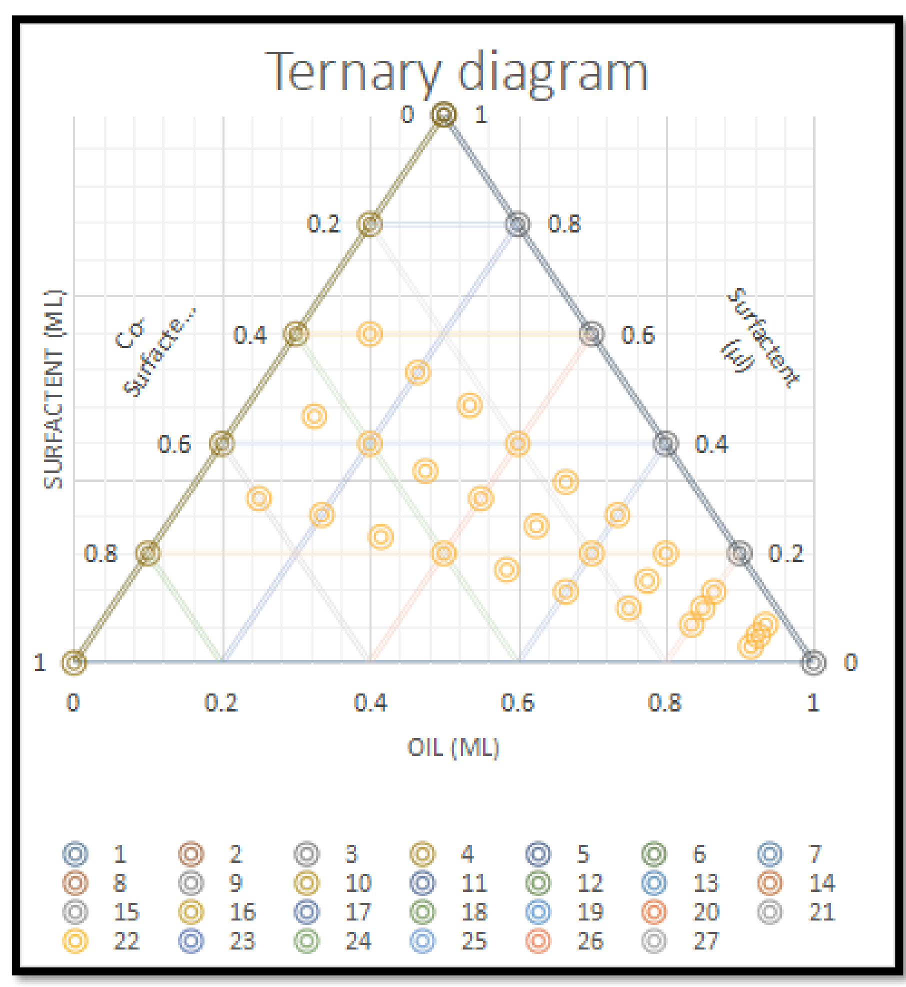
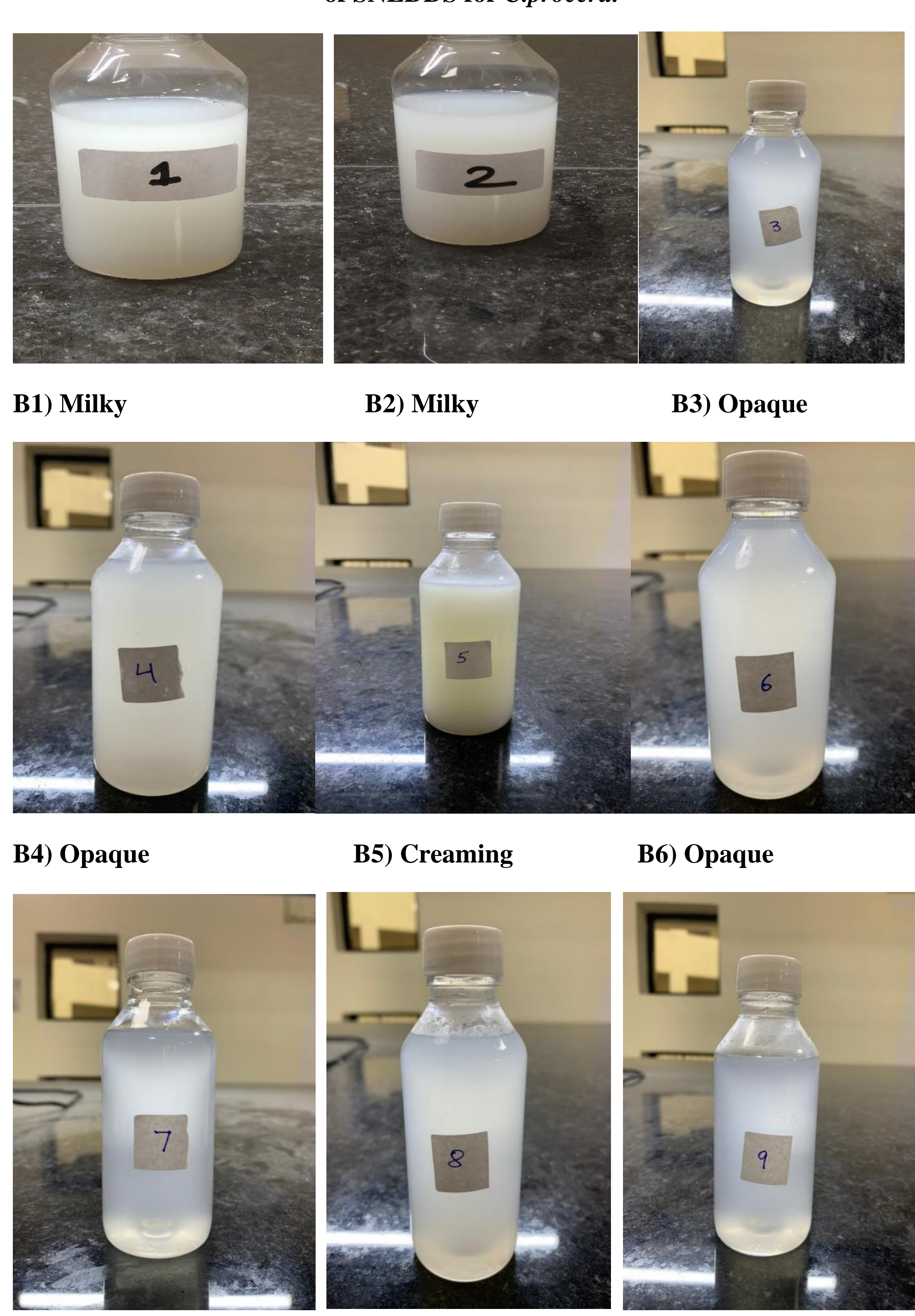


Figure 6.2-Ternary phase diagram

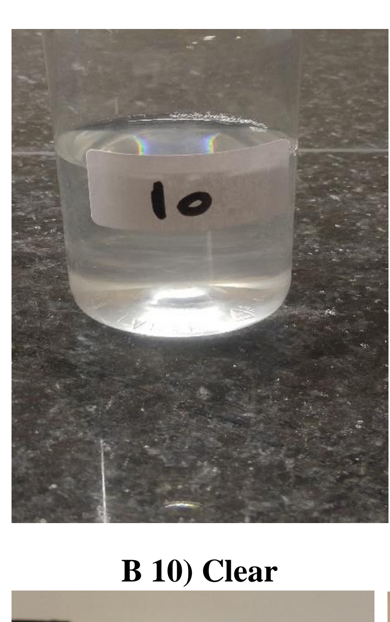
Figure.6.3- Apperance of different concentrations prepared for the development of SNEDDS for *C.procera.*



B1) Milky **B2) Milky** **B3) Opaque**

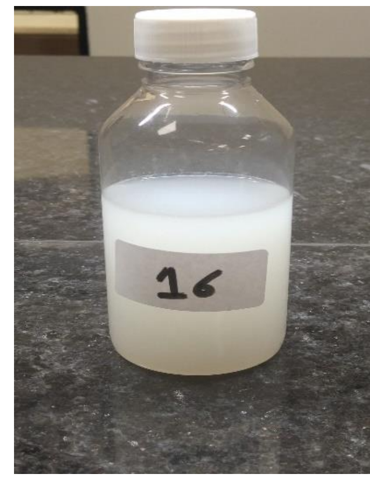
B4) Opaque **B5) Creaming** **B6) Opaque**

B7) Turbid **B8) Turbid** **B9) Opaque**



B 10) Clear B11) Turbid B12) Turbid

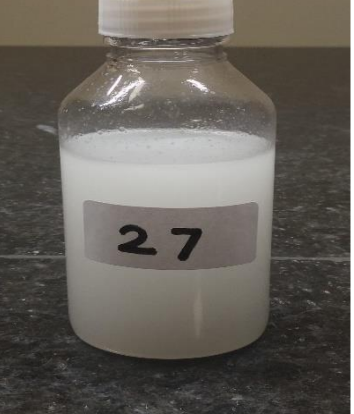
B13) Turbid B14) Turbid B15) Creaming



B16) Creaming B17) Creaming B18) Turbid

B19) Clear **B20) Milky** **B21) Turbid**

B22) Turbid **B23) Opaque** **B24) Turbid**



B25) Turbid **B26) Creaming** **B27) Creaming**

6.9 Characterization of SNEDDS[67,68]:

6.9.1 Entrapment efficiency[69]: SNEDDS were centrifuged and the proportion of encapsulated drugs was determined. Appropriate dilution was made in order to measure the content using UV spectrophotometry at 269. The entrapment efficiency was calculated 90.7%. for the prepared SNEDDS.

6.9.2 Transmission electron microscopy (TEM): TEM imaging of SNEDDS was evaluated under high resolution to determine the particle size of the.

Droplet size of the optimised batch of SNEDDS was observed as 50 nm and droplets were spherical in shape as shown in the image 6.4.

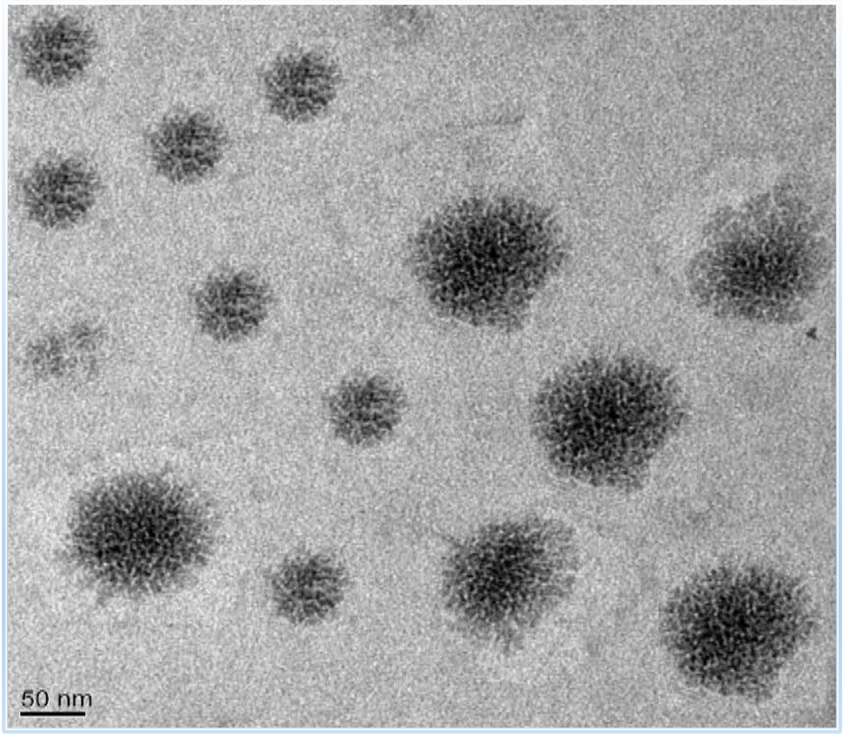


Image.6.4: Transmission electron microscopy of SNEDDS of *C. procera*

6.9.3 Zeta potential[70]: No charge in the microemulsion droplet was found as the resulted Zeta potential was -12.3 mv, which indicate that the microemulsion droplets had no charge and lying under the acceptable range of +25mV to -25mV.The result is shown in figure 6.3.

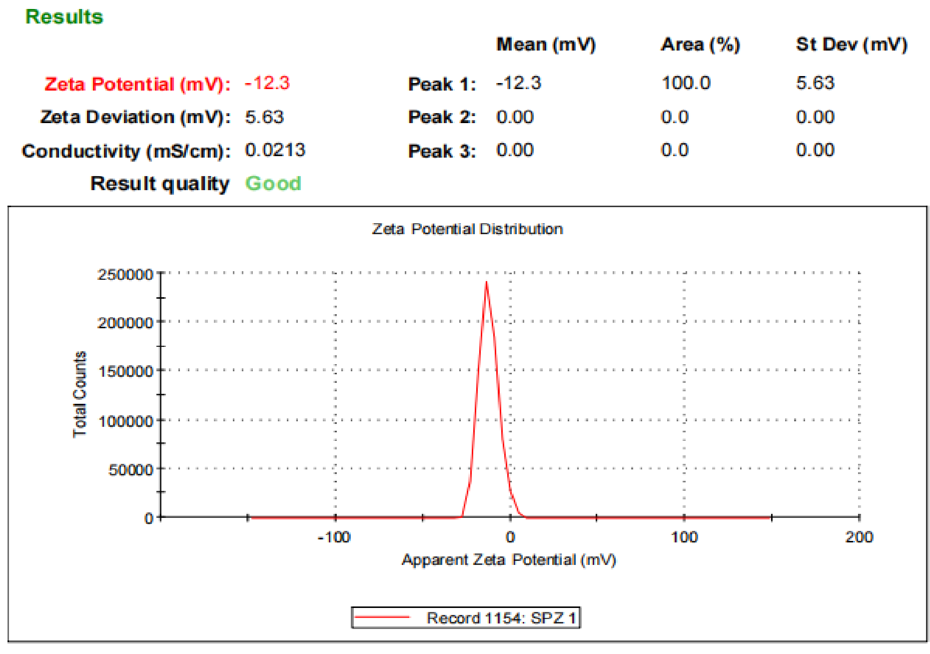


Figure 6.3- Zeta potential of SNEDDS of *C. procera*

6.9.4 Droplet size and Polydispersity Index[71,72]: The PDI was determined by using Malvern Nano Zeta Seizer instrument. PDI reflects the uniformity of droplet diameter. The average droplet size and polydispersity index lying in the nano range of 153 nm and 0.517 respectively. The result is shown in figure 6.4.

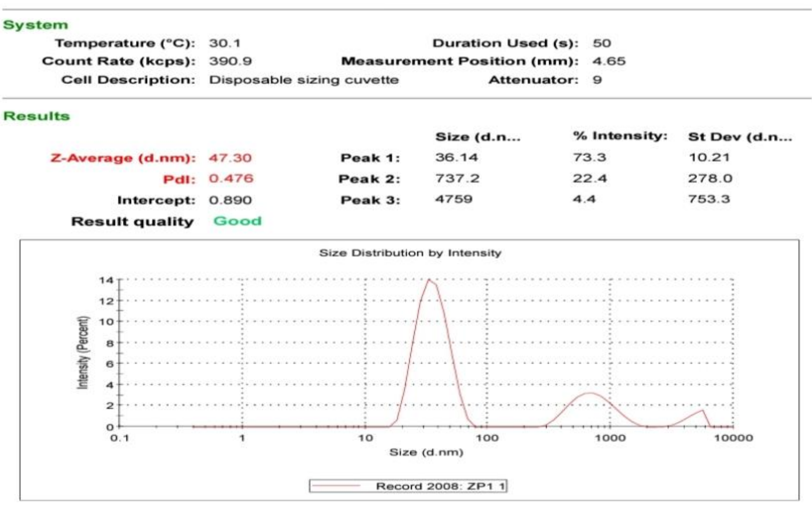


Figure 6.4- Droplet size and Polydispersity Index of SNEDDS of *C. procera*

6.9.5 Dissolution Studies[74]

The dissolution of drug from SNEDDS and extract were shown in figure number 6.5 and it was observed that SNEDDS formulation allowed 99% dissolution in 90 min. and 32 % for that of extract in 90 min. The dissolution rate of SNEDDS formulation was observed better and faster than extract, this is due to the fact of small droplet size of SNEDDS. In the pure extract solution, 32.065±0.164% was released within 90 mins and *in-vitro* %age of extract released from SNEDDS was founded 99.783±0.652%. %age of drug released was calculated from absorbance and concentration that were obtained with the help of standard graph. *In-Vitro* drug release of SNEDDS & Pure extract was shown in figure 6.5 and percentage drug release of SNEDDS and percentage drug release of extract was shown in table 6.14

Table 6.14: %age Drug release of SNEDDS and %age Drug release of extract

Sr.no	Time (Min.)	Drug Release of SNEDDS (%)	Drug Release of Extract (%)
1	0	0	0
2	5	52.931±0.753	9.739±0.199
3	10	87.261 ± 0.897	13.478 ±0.458
4	15	97.609 ±0.377	16.361 ±0.196
5	30	99.152 ±0.621	21.609 ±0.075
6	45	99.348 ±0.753	25.452 ±0.316
7	60	99.783 ±0.652	29.443 ±0.060
8	90	99.829 ±0.665	32.065 ±0.164

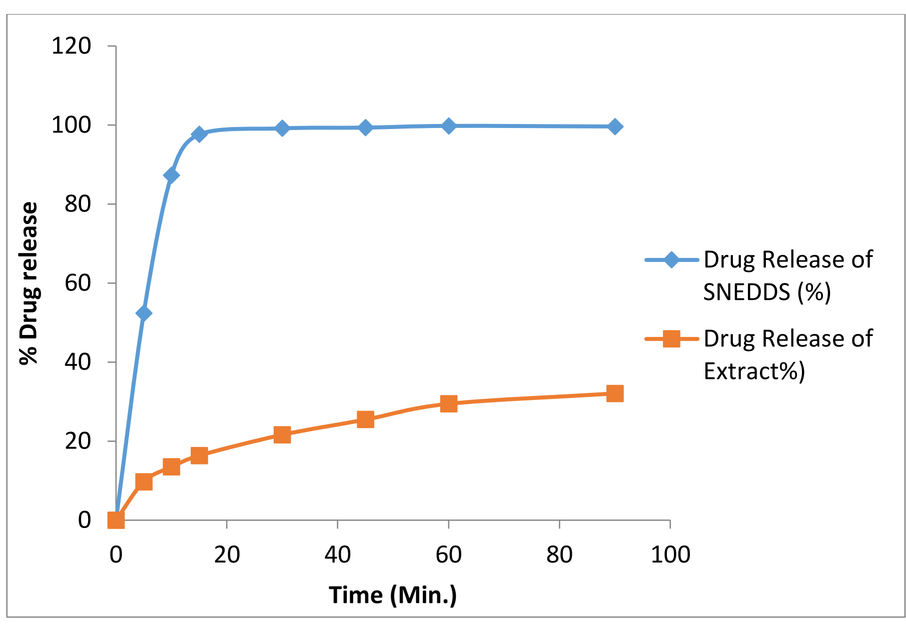


Figure 6.5- *In-Vitro* drug release SNEDDS & C.P extract

6.9.6 Accelerated Stability Study: The study used to predict the stability of compounds of the formulation at long term storage conditions which results significant benefit in the product development. The degradation of the product in a given time period can be noticed through accelerated stability studies. Through accelerated stability studies investigation is being done for physical and oxidative stability of the sample. Accelerated studies are a common approach for predicting the long-term stability of the formulation. Organoleptic, physical and microbial analysis were checked for the optimised batch of SNEDDS of C.P. extract. The organoleptic characteristic i.e., description and odour were analysed for 3 months and 6 months. The optimised batch remain clear for the given period of time. Physio-chemical properties of optimised batch show no change in specific gravity, pH and viscosity for 6 months. Sample was subjected for microbial analysis to investigate the growth of gram positive and gram negative bacteria and the range comes in the prescribed limit as per monograph. There was no change observed for 6 months in the optimised batch of SNEDDS of C.P. extract. The result was shown in table 6.18. HPTLC fingerprinting was evaluated and it was observed that all the R_f found at different absorbance were almost same except 0.17 in table.6.15

Table - 6.15 Rf value 254 nm absorbance

Spot no.	Track T1	Track T2
1	-	0.17
2	0.21	0.21
3	0.43	0.43
4	0.49	0.49
5	0.59	0.59
6	0.68	0.68
7	0.89	0.86

Track T1: SNEDDS (*Calotropis procera*)- 0 month

Track T2: SNEDDS (*Calotropis procera*)- 6 month

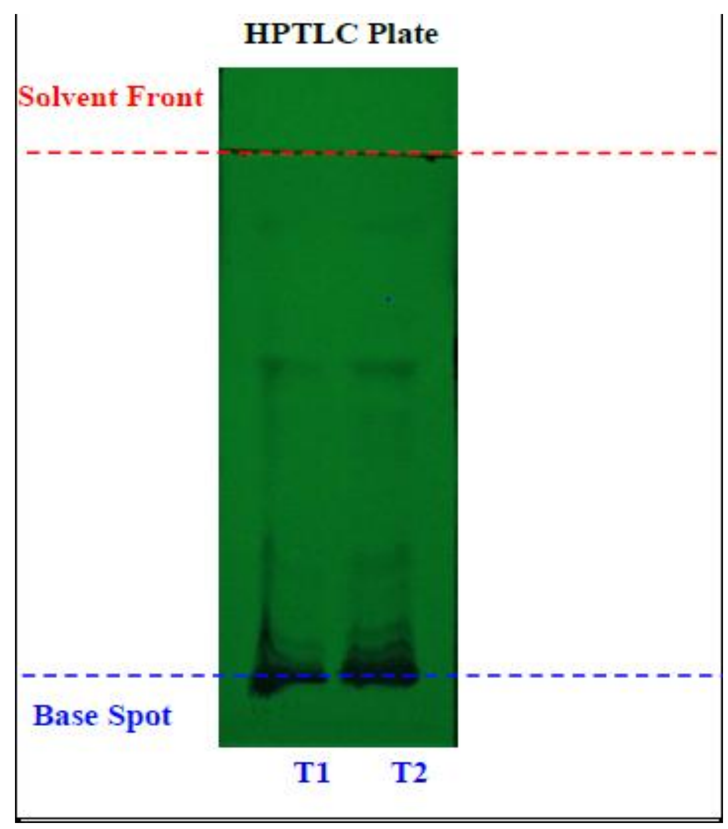


Image 6.5-HPTLC fingerprinting 254 nm absorbance

Table 6.16 Rf value 366 nm absorbance

Spot no.	Track T1	Track T2
1	0.68	0.68
2	0.73	0.73
3	0.81	0.81

Track T1: SNEDDS (*Calotropis procera*)- 0 month

Track T2: SNEDDS (*Calotropis procera*)- 6 month

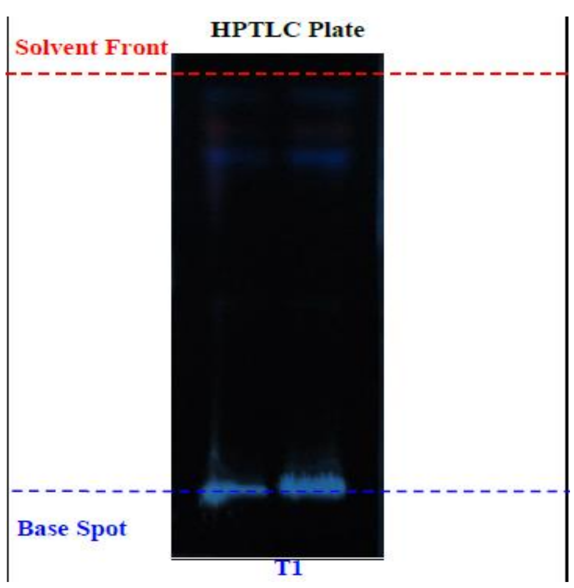


Image 6.6- HPTLC fingerprinting 366 nm absorbance

Table no. 6.17 Rf value 540 nm absorbance

Spot no.	Track T1	Track T2
1	0.17	0.17
2	0.21	0.21
3	0.26	0.26
4	0.38	0.38
5	0.49	0.49
6	0.59	0.59
7	0.64	0.64
8	0.81	0.81
9	0.86	0.86

Track T1: SNEDDS (*Calotropis procera*)- 0 month

Track T2: SNEDDS (*Calotropis procera*)- 6 month

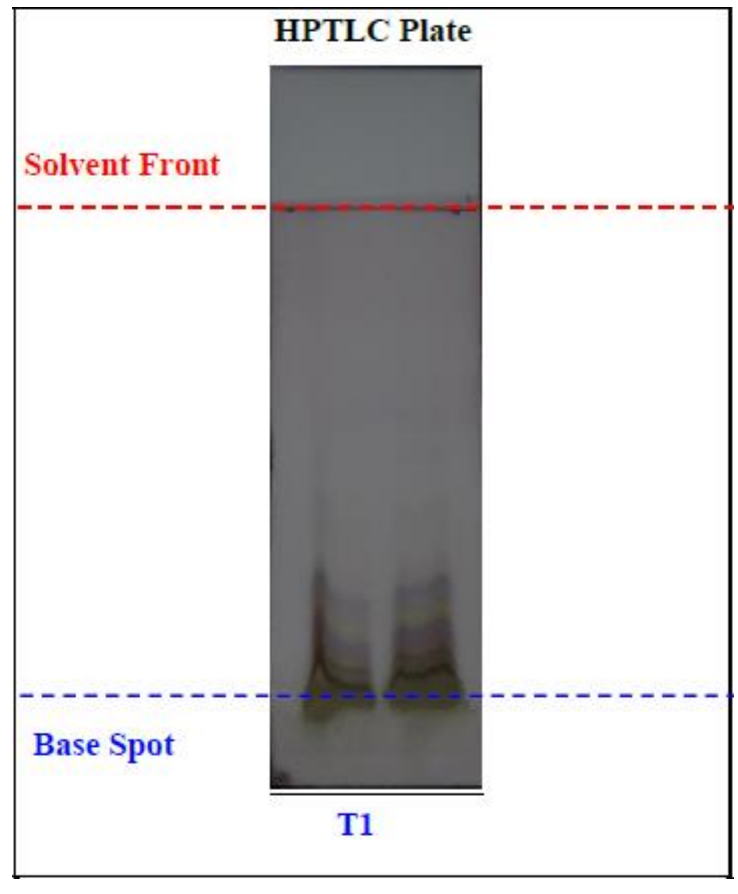


Image 6.7- HPTLC fingerprinting 540 nm absorbance

Table 6.18- Accelerated Stability Study of the optimised batch of SNEDDS

Sr. No.	Parameters	0 Month	3rd Month	6th Month
	Organoleptic analysis			
1	Description	Clear liquid	Clear liquid	Clear liquid
2	Odour	Characteristic	Characteristic	Characteristic
		Physico-chemical analysis		
1	Specific gravity	1.017	1.021	1.025
2	pH	3.31	3.32	3.35
3	Viscosity	0.62 cP	0.64 cP	0.68 cP

MICROBIAL ANALYSIS				
1	Total microbial load Count	< 10 cfu/g	-	< 10 cfu/g
2	Total Yeast & Mould Count		ND	ND
3	Staphylococcus aureus		ND	ND
4	Salmonella sp.		ND	ND
5	Pseudomonas aeruginosa		ND	ND
6	Escherichia coli		ND	ND

(ND – Not detected)

6.10 *In- vitro* studies[75,76]

6.10.1 A) Anti-oxidant activity[77]:

1) 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH assay):

The DPPH activity value for standard was found 138.78. For extract IC_{50} value was found to be 100.08 and for formulation IC_{50} value was found 67.80 respectively. The study was repeated in triplicate. Result is shown in table 6.19 and image 6.8.

Table 6.19- DPPH assay of Standard, Extract and Formulation

Conc. (µg/ml)	% inhibition of Standard	% inhibition of extract	% inhibition of formulation
20	13.29±1.86	30.33±2.65	37.33±2.63
40	22.15±2.78	38.76±2.16	45.76±2.72
60	30.37±2.97	42.66±3.1	49.66±1.82
80	34.17±2.88	46.85±1.89	53.85±3.12
100	36.07±1.95	49.54±2.17	56.54±2.87
120	44.65±2.44	52.66±2.55	59.66±1.93

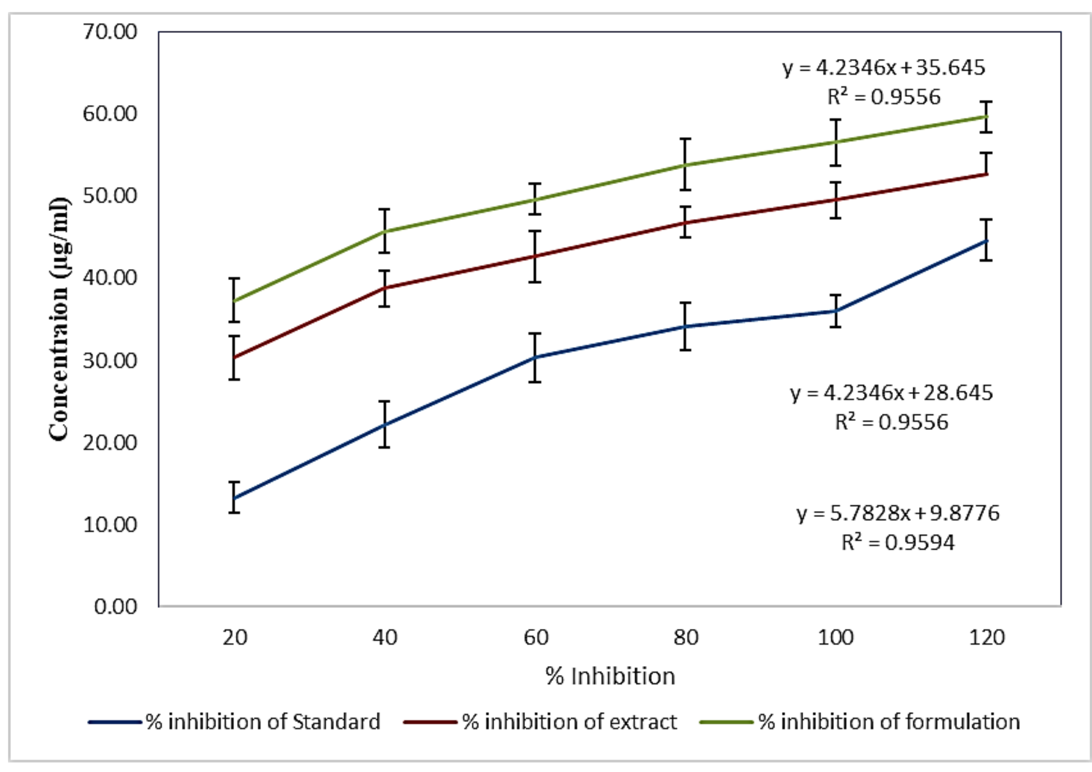


Image 6.8 - DPPH radical scavenging activity of standard, SNEDDS and extract of *C. procera*. Data expressed as mean ± s.d.

Different concentrations of standard, SNEDDS and extract was used to check the anti-oxidant activity by performing DPPH assay. %inhibition of SNEDDS were high as compared to extract. IC_{50} value of SNEDDS, extract and standard clearly describes the better antioxidant activity of SNEDDS whe compared to the ethanolic extract of C.P. and standard used for the experiment. Different observation of the DPPH assay was mentioned in table 6.19 and image 6.8 showing the graph between the different concentrations and absorbance of all the samples.

2) Hydrogen peroxide (H_2O_2) scavenging assay[78]:

The observed IC_{50} value for Standard was found 194.69. For extract IC_{50} value was found to be 136.42 and for Formulation IC_{50} value was found 93.26 respectively. The study was repeated in triplicate. Result is shown under table 6.20 and image 6.9.

Table 6.20- Hydrogen Peroxide scavenging assay

Conc. (µg/ml)	% inhibition of Standard	% inhibition of extract	% inhibition of formulation
20	11.07±1.67	25.27±2.02	32.27±2.36
40	18.46±2.13	32.30±1.88	39.30±1.85
60	25.31±2.71	36.69±2.12	43.69±2.75
80	28.48±2.63	40.65±3.13	47.65±2.22
100	30.06±2.11	43.07±2.67	50.07±2.88
120	32.34±1.97	45.05±2.59	56.05±3.11

Image 6.9- Hydrogen peroxide scavenging activity of standard, SNEDDS and extract of *C. procera*. Data expressed as mean ± Sc.D.

Different concentration of standard, SNEDDS and extract were used to check its anti-oxidant activity by performing Hydrogen peroxide scavenging activity. % inhibition of SNEDDS were high as compared to extract. IC_{50} value of SNEDDS, extract and standard clearly describes the better antioxidant activity of SNEDDS when compared to the ethanolic extract of C.P. and standard used for the experiment.

6.10.2 B) α-amylase inhibition assay[79,80]:

The observed IC_{50} value for standard was found 56.85. For extract IC_{50} value was found to be 32.07 and formulation IC_{50} value was found 20.44 respectively. The study was repeated in triplicate.

Table 6.21 α-amylase inhibition assay

Conc. (µg/ml)	% inhibition of Standard	% inhibition of extract	% inhibition of formulation
20	26.57±2.89	45.49±1.93	55.49±1.89
40	38.30±2.73	58.14±2.16	68.14±2.42
60	50.66±1.99	66.05±2.81	76.05±2.78
80	59.32±2.98	73.18±1.86	86.18±3.21
100	84.72±3.16	93.54±1.86	119.54±2.47
120	96.52±1.89	117.54±3.02	136.54±1.94

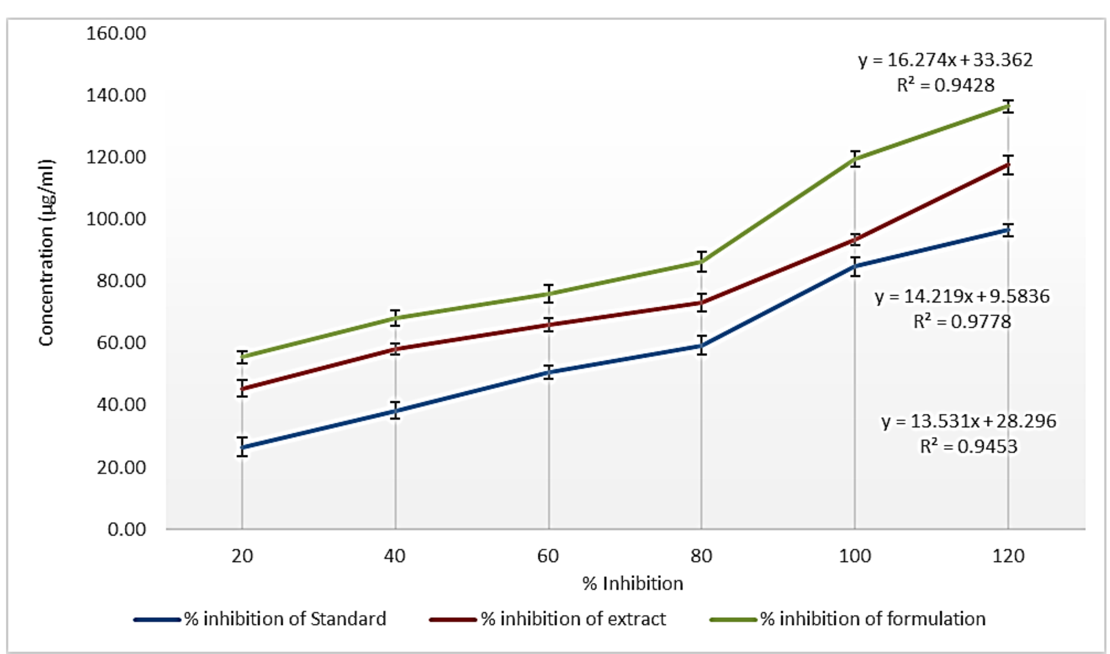


Image 6.10- α- amylase assay of standard, SNEDDS and ethanolic extract of *C. procera*. Data was expressed as mean ± Sc.D.

Different concentration of standard, SNEDDS and extract were used to check its anti-diabetic activity by performing α-amylase assay. Percent inhibition of SNEDDS were high as compared to extract. IC50 value of SNEDDS, extract and standard clearly describes the better antidiabetic activity of SNEDDS when compared to the ethanolic extract of C.P and standard used for the study. Different observation of the α- amylase assay is mentioned in table 6.21 and fig.6.10 showing the graph between the different concentrations and absorbance of all the samples.

6.11: *In-vivo* studies:

Results

6.11.1 Body weight

Body weight of animal measurement is first and important parameter for further evaluation of the prepared formulation. Weight of the rats were checked on 1st, 15th, 22nd and 29th day of the experiment. on 15th day significant increase in body weight was observed in rats fed with high fat diet, as compared to rats fed with normal pellet diet. No significant difference was observed in CP extract *per se* group and SNEDDS *per se* group, when compared to vehicle control group. On 22nd day after one week of STZ injection, no significant difference was observed all the NPD fed groups in comparison to the vehicle control. On 22nd day, it was observed that loss in body weight occur in the groups with HFD diet due to development of diabetes. On 29th day; no significant difference observed in body weight of rats in CP extract *per se* and SNEDDS *per se* treated group, when compared with vehicle control group. Significant decrease in body weight was observed in standard treated and SNEDDS 100 mg/kg dose treated groups, as compared to disease control while no significant difference of bodyweight was observed in remaining groups while comparing with disease control group. Significant increase in body weight was observed in CP extract 400 mg/kg and CP SNEDDS 200 mg/kg treated groups, when compared to standard treated group. Different treatment observation on body weight of rats was shown in table 6.22 and figure 6.7

Table 6.22- Various treatments effects on body weight of rats

Group name	1st day	15th day	22nd day	29th day
Vehicle Control	242.5 ± 19.64	254.16 ± 10.41	261.33 ± 10.57	258.83 ± 9.74
CP extract per se	243.5 ± 9.97	253 ± 10	252.66 ± 8.06	257.16 ± 12.89
SNEDDS per se	242.16 ± 10.18	253.16 ± 9.15	256.16 ± 9.41	261 ± 5.76
Disease control	245.85 ± 7.19	289.57 ± 18.59***	273 ± 10.34	266.42 ± 5.28
Acarbose + STZ + HFD	244 ± 10.45	279.71 ± 9.28*	265.28 ± 9.03	229.28 ± 11.70###
CP extract 200 mg/kg + STZ + HFD	250.85 ± 7.53	289.42 ± 11.70***	269.85 ± 14.07	254.85 ± 14.94^^
CP extract 400 mg/kg + STZ + HFD	248 ± 13.25	277.28 ± 14.53*	263.71 ± 9.89	268.57 ± 13.95^^^
CP extract SNEDDS 100 mg/kg + STZ + HFD	250.28 ± 14.53	294.71 ± 12.35***	273.42 ± 15.50	247.42 ± 8.42#
CP extract SNEDDS 200 mg/kg + STZ + HFD	250.85 ± 13.90	284.14 ± 9.20**	264.57 ± 13.33	262.42 ± 10.69^^^

Data presented as mean ± SD; *, **, *** describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with vehicle control group; #, ##, ### describes $p<0.05$, $p<0.01$, $p< 0.001$, respectively, when compared with negative control group and ^, ^^, ^^^ describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with positive control.

Vehicle Control
CP extract per se
SNEDDS per se
Disease control
Acarbose + STZ + HFD
CP extract 200 mg/kg + STZ + HFD
CP extract 400 mg/kg + STZ + HFD

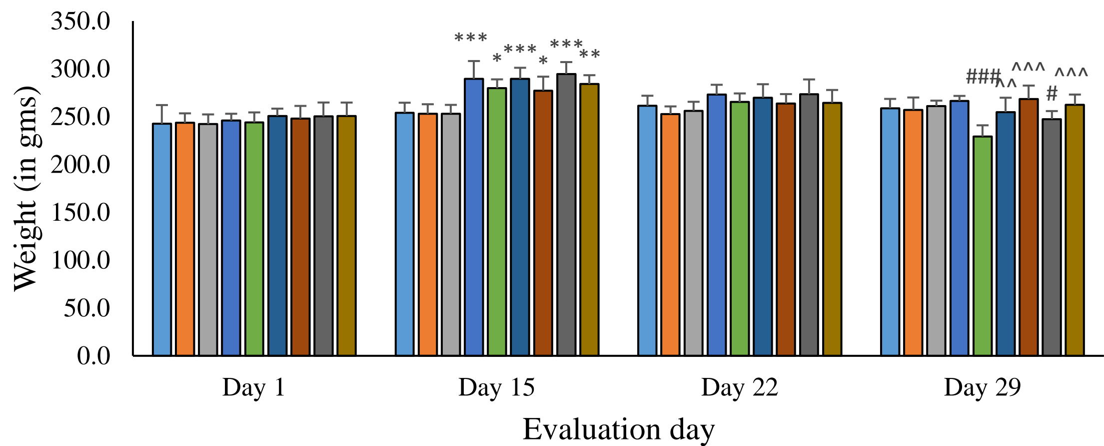


Figure 6.6 – Effect of different treatments on body weight of rats. Data presented as mean ± SD; $^{*, **, ***}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with vehicle control group; $^{\#, \#\#, \#\#\#}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with negative control group and $^{\wedge, \wedge\wedge, \wedge\wedge\wedge}$ represents p<0.05, p<0.01, p<0.001, respectively, when compared with positive control.

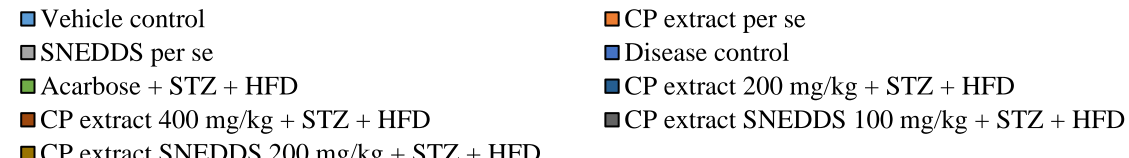
6.11.2 Plasma glucose (PGL)

Plasma glucose level was evaluated on 22nd day and 29th day of the experiment. On 22nd day of the experiment, significant increase was seen in PGL level in all the HFD + STZ treated groups in comparison to the control which confirm the development of type 2 diabetic condition. On 29th day, significant increased level of PGL was observed in disease control group, as compared to vehicle control group. Increased in the level of PGL was significantly reversed with standard drug treatment, CP extract treatment (low and high dose) and CP extract SNEDDS treatment (low and high dose), when it was compared to disease control group. Effect of different treatments on PGL were shown in table 6.23 and figure 6.7.

Table 6.23- Effect of different treatments on PGL

Group Name	22nd day	29th day
Vehicle control	82.2±7.52	82.0±7.16
CP extract per se	86.2±8.38	87.3±7.50
SNEDDS per se	86.2±9.66	85.3±9.85
Disease control	340.7±9.96***	330.9±15.75***
Acarbose + STZ + HFD	345.9±6.09***	229.0±9.47###
CP extract 200 mg/kg + STZ + HFD	333.0±13.82***	277.1±18.72###
CP extract 400 mg/kg + STZ + HFD	341.1±13.83***	265.7±16.93###
CP extract SNEDDS 100 mg/kg + STZ + HFD	330.7±12.45***	208.7±8.38###
CP extract SNEDDS 200 mg/kg + STZ + HFD	340.0±10.52***	196.7±8.14###

Data is presented as mean ± SD. *, **, *** describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with vehicle control group; #, ##, ### describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with negative control group.



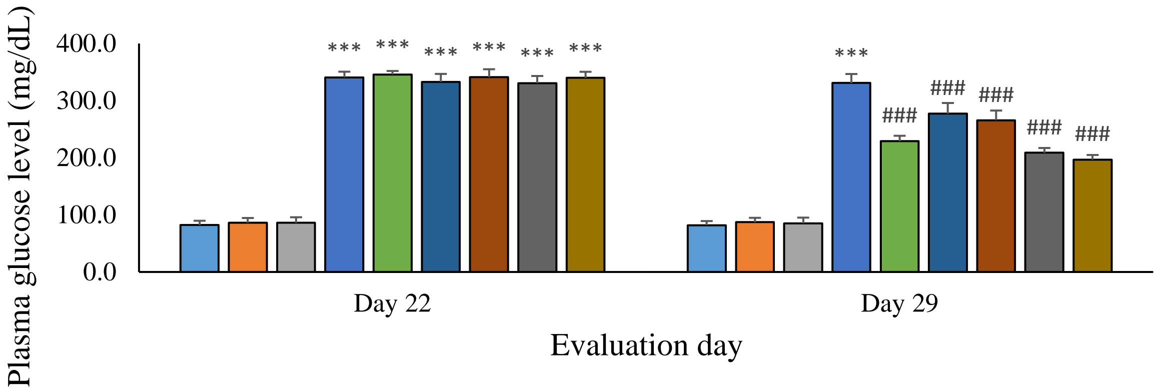


Figure 6.7- Effect of different treatments on PGL. Data is presented as mean ± SD. *, **, *** describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with vehicle control group; #, ##, ### describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with negative control group.

6.11.3 Plasma total cholesterol

The plasma total cholesterol was evaluated on 22nd day and 29th day of the experiment. On 22nd day of the experiment; there is significant increase was seen in PTC level in all the HFD + STZ treated groups in comparison to the disease control group which confirm the type 2 diabetic condition. On 29th day, significant increased levels of PTC were observed in disease control group, as compared to vehicle control group. This significant increase in PTC levels were significantly reversed by the treatment with standard drug treatment, CP extract treatment (high dose), and CP extract SNEDDS treatment (low and high dose), when compared to disease control group. CP extract SNEDDS treatment (low and high dose) significantly improved the PTC levels of rats, as compared to acarbose treated positive control group. The effects of respective treatments were shown in table 6.24 and figure 6.8.

Table 6.24- PTC level treatment effects

Group Name	22nd day	29th day
Vehicle control	38.2±6.74	42.0±4.86
CP extract per se	40.2±6.79	38.3±3.98
SNEDDS per se	36.3±6.25	36.0±4.56
Disease control	122.1±10.46***	142.1±2.53***
Acarbose + STZ + HFD	118.1±9.56***	105.0±5.45$^{\#\#\#}$
CP extract 200 mg/kg + STZ + HFD	118.7±4.54***	122.1±8.84$^{\#\#\#}$
CP extract 400 mg/kg + STZ + HFD	117.1±11.77***	112.1±7.63$^{\#\#\#}$
CP extract SNEDDS 100 mg/kg + STZ + HFD	122.7±7.13***	87.3±11.13$^{\#\#\#, \wedge\wedge\wedge}$
CP extract SNEDDS 200 mg/kg + STZ + HFD	123.1±9.42***	73.4±8.66$^{\#\#\#, \wedge\wedge\wedge}$

Table 6.24 – Data is represented as mean ± SD. $^{*, **, ***}$ describes $p<0.05$, $p<0.01$, $p<0.001$, describes, when compared with vehicle control group; $^{\#, \#\#, \#\#\#}$ describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with negative control group and $^{\wedge, \wedge\wedge, \wedge\wedge\wedge}$ describes $p<0.05$, $p< 0.01$, $p < 0.001$, respectively, when compared with positive control.

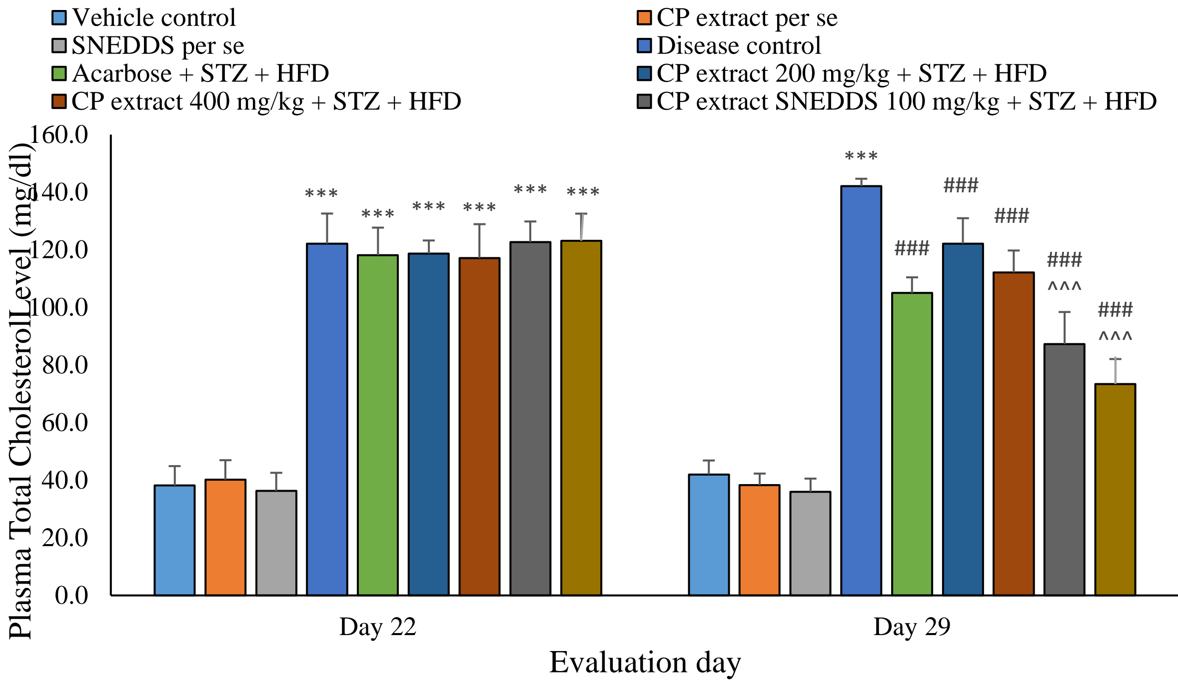


Figure 6.8- Effect of different treatments on plasma cholesterol levels Data is describes as mean ± SD. *, **, *** describes p<0.05, p<0.01, p< 0.001, respectively, when compared with vehicle control group; #, ##, ### describes p<0.05, p<0.01, p<0.001, respectively, when compared with negative control group and ^, ^^, ^^^ describes p<0.05, p<0.01, p<0.001, respectively, when compared with positive control.

6.11.4 Thiobarbituric acid reactive substances estimation

Thiobarbituric acid reactive substances in rat's pancreas was estimated on 29th day of the experiment. No significant difference was observed after treatment with CP extract *per se* and SNEDDS *per se* treated group, when compared to vehicle control group. Increase in the levels of TBARS were observed in negative control group when compared to vehicle control group. This increased levels of thiobarbituric acid reactive substances were significantly reversed by the treatment with standard drug treatment, CP extract treatment (low and high dose), and CP extract SNEDDS treatment (low and high dose), when compared to disease control group. CP extract treatment (low and high dose) and CP extract SNEDDS treatment (low and high dose) significantly improved the TBARS levels of rats, as compared to acarbose treated positive control

group. Best effect was observed in CP extract SNEDDS high dose treated group. Effects of respective treatments were shown in table 6.25 and figure 6.9

6.11.5 Estimation of reduced glutathione (GSH)

GSH in rat's pancreas was estimated on 29th day of the study. No significant difference was observed in GSH levels after treatment with CP extract *per se* and SNEDDS *per se* treated group, when compared to vehicle control group. Significantly decreased levels of GSH were observed in negative control group, as compared to vehicle control group. This decreased levels of GSH were significantly reversed by the treatment with standard drug treatment, CP extract treatment (low and high dose), and CP extract SNEDDS treatment (low and high dose), when compared to disease control group. CP extract treatment (low and high dose) and CP extract SNEDDS treatment (low and high dose) significantly improved the GSH levels of rats, as compared to acarbose treated positive control group. Best effect was observed in CP extract SNEDDS high dose treated group. Effects of respective treatments were shown in table 6.25 and figure 6.10

6.11.6 Estimation of catalase (CAT) activity

CAT in rats' pancreas was estimated on 29th day of the study. No significant difference was observed in GSH levels after treatment with CP extract *per se* and SNEDDS *per se* treated group, when compared to vehicle control group. Significantly decreased levels of CAT were observed in negative control group, as compared to vehicle control group. These decreased levels of CAT were significantly reversed by the treatment with standard drug treatment, CP extract treatment (low and high dose), and CP extract SNEDDS treatment (low and high dose), when compared to disease control group. CP extract treatment (high dose) and CP extract SNEDDS treatment (low and high dose) significantly improved the CAT levels of rats, as compared to acarbose treated positive control group. Best effect was observed in CP extract SNEDDS high dose treated group. Effects of respective treatments were shown in table 6.25 and figure 6.11

Table 6.25- Effect of different treatments on TBARS, GSH and CAT activity of rat pancreas

Group Name	TBARS (nM/mg protein)	GSH (µM/mg protein)	CAT (mM/mg protein)
Vehicle control	0.5±0.06	1.8±0.05	1.9±0.06
CP extract per se	0.4±0.04	1.9±0.06	2.0±0.06
SNEDDS per se	0.5±0.06	1.7±0.07	1.9±0.07
Disease control	1.3±0.07***	0.8±0.09***	0.7±0.06***
Acarbose + STZ + HFD	0.9±0.03###	1.2±0.08###	1.4±0.07###
CP extract 200 mg/kg + STZ + HFD	0.8±0.04###, ^^^	1.4±0.05###, ^^^	1.4±0.09###
CP extract 400 mg/kg + STZ + HFD	0.7±0.06###, ^^^	1.4±0.06###, ^^^	1.7±0.09###, ^^^
CP extract SNEDDS 100 mg/kg + STZ + HFD	0.7±0.05###, ^^^	1.4±0.10###, ^^^	1.7±0.06###, ^^^
CP extract SNEDDS 200 mg/kg + STZ + HFD	0.6±0.06###, ^^^	1.5±0.06###, ^^^	1.8±0.08###, ^^^

Data is represented as mean ± SD, *, **, *** describes p<0.05, p<0.01, p<0.001, respectively, when compared with vehicle control group; #, ##, ### describes p<0.05, p<0.01, p<0.001, respectively, when compared with negative control group; and ^, ^^, ^^^ describes p<0.05, p<0.01, p<0.001, respectively, when compared with positive control.

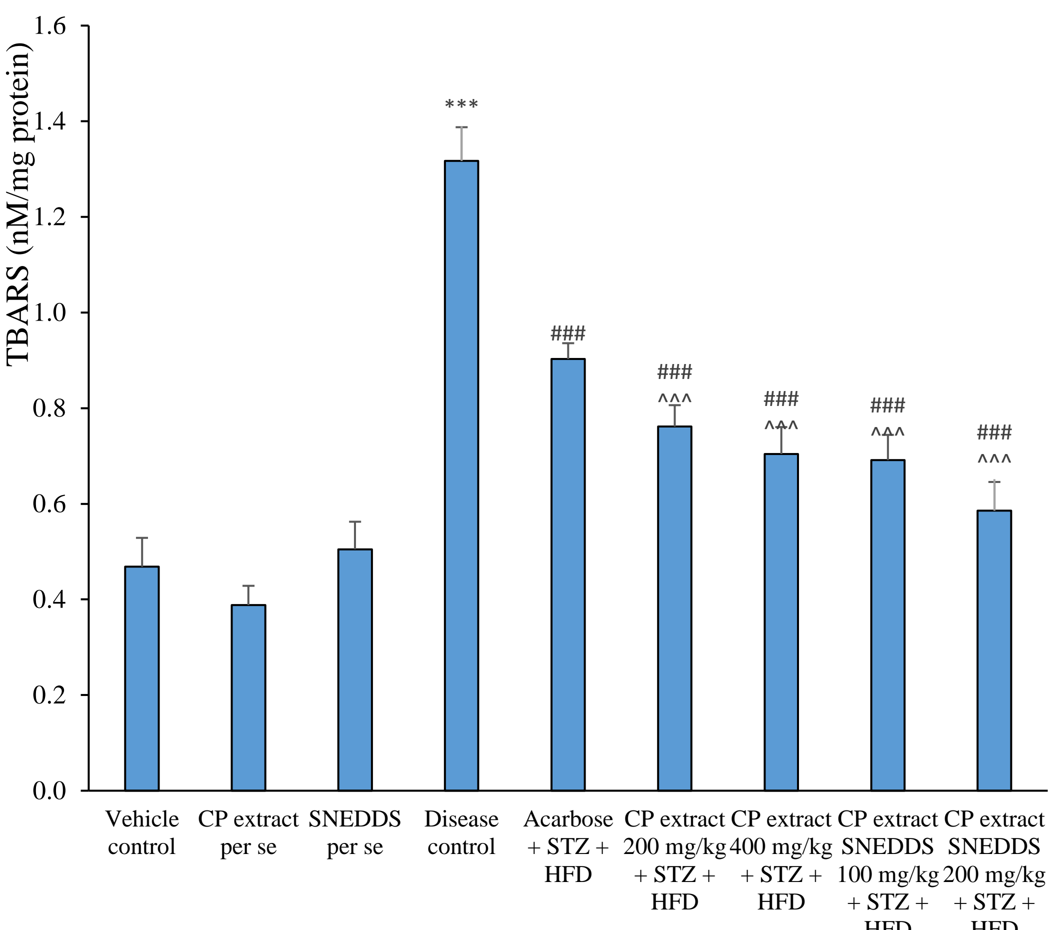


Figure 6.9-Effect of different treatments on TABRS activity of rat pancreas. Data describes as mean ± SD. $^{*, **, ***}$ represents p<0.05, p<0.01, p<0.001, respectively, when compared with vehicle control group; $^{\#, \#\#, \#\#\#}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with negative control group and $^{\wedge, \wedge\wedge, \wedge\wedge\wedge}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with positive control.

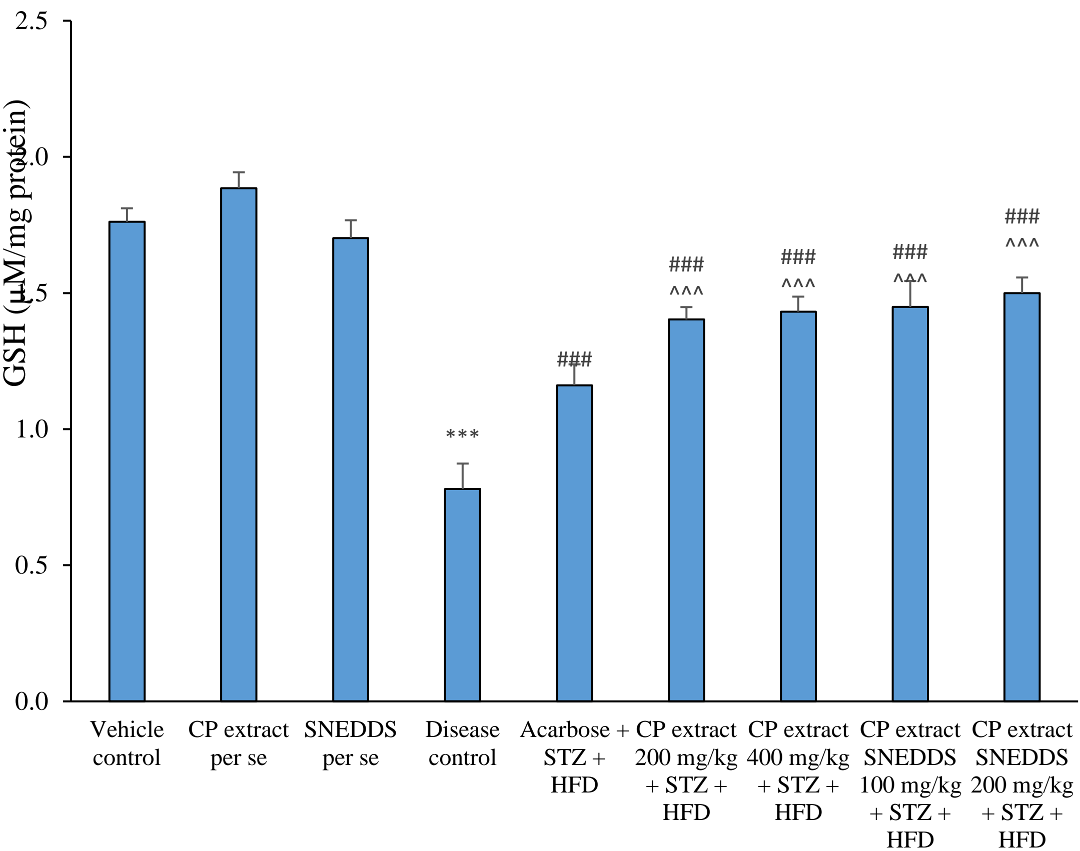


Figure 6.10-Effect of different treatments on GSH activity of rat pancreas. Data is represented as mean ± SD. $^{*, **, ***}$ describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with vehicle control group; $^{\#, \#\#, \#\#\#}$ describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with negative control group; and $^{\wedge, \wedge\wedge, \wedge\wedge\wedge}$ describes $p<0.05$, $p<0.01$, $p<0.001$, respectively, when compared with positive control.

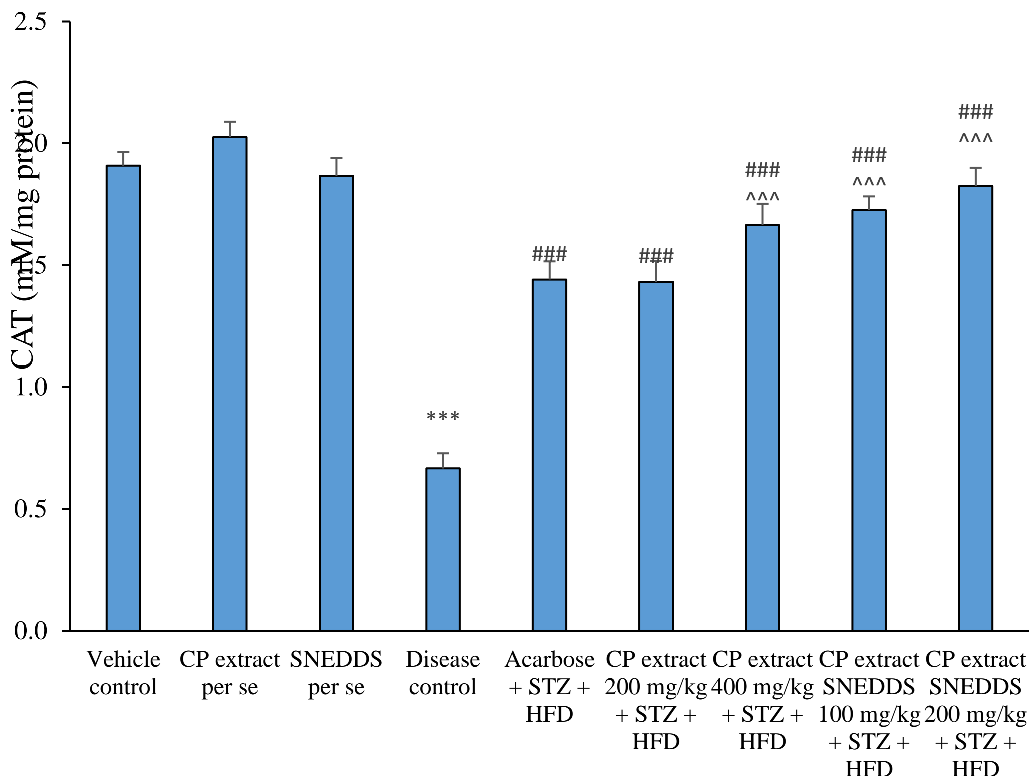


Figure 6.11-Effect of different treatments on CAT activity of rat pancreas. Data describes as mean ± SD. *, **, *** represents p<0.05, p<0.01, p<0.001, respectively, when compared with vehicle control group; $^{\#}$, $^{\#\#}$, $^{\#\#\#}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with negative control group and $^{\wedge}$, $^{\wedge\wedge}$, $^{\wedge\wedge\wedge}$ describes p<0.05, p<0.01, p<0.001, respectively, when compared with positive control.

In-Vivo results and discussion

Animal study was done to investigate the results of the C.P SNEDDS and C.P extract group in comparison to negative and positive control group. Rats were divided into total 9 groups total i.e., vehicle, CP extract *per se,* SNEDDS *per se,* disease control, standard control, high and low dose of C.P extract and high and low dose of C.P SNEDDS. High fat diet was administered to the disease control, standard control, high and low dose of C.P extract and high and low dose of C.P SNEDDS for consecutive 28 days. Body weight was calculated on 1st day, 15th day, 22nd day and 29th day of the experiment (results shown in table no.6.22 and figure no. 6.6). Increase in the body weight was observed on 15th day of the experiment in groups feeding with HFD diet. On 15th day streptozotocin was administered to HFD group and again weighed on 22nd day which shows decrease in body when compared to NPD diet feeding rats. This was due to the fact that diabetes was induced after administration of streptozotocin. On 29th day, no difference in the weight was observed in vehicle, CP extract *per se,* SNEDDS *per se* groups, when compared with disease control group. Group with 200 mg extract and SNEDDS with 100 mg extract has shown no significant effect when compared to standard group. This effect was reversed on groups treated with high dose of SNEDDS and CP extract with high dose as decrease in weight was not observed, when compared to standard group.

Plasma glucose level and plasma total cholesterol level, both were estimated on 22nd and 29th day of the study. No effect was shown in CP extract *per se,* SNEDDS *per se* group when compared to vehicle group. Plasma glucose level was increased in negative control group when compared with vehicle group. This increased level of plasma glucose was reversed in the groups treated with low and high dose of C.P extract group and low and high dose of SNEDDS group, when compared to disease control group as shown in table no. 6.23 and figure no. 6.7. Increase in plasma total cholesterol levels in all groups treated with streptozotocin was observed on 22nd day of the study and no change in vehicle control group was observed. On 29th day after 7 days treatment, increase in plasma total cholesterol was observed in disease control group as compared to vehicle group results as shown in table no. 6.24 and figure no. 6.8. Reversed effect

on plasma total cholesterol level was observed in the groups treated with low and high dose of CP extract and low and high dose of SNEDDS. Improvement in the plasma total cholesterol level of rats was observed when compared to positive control group treated with acarbose.

Thiobarbituric acid reactive substances (TBARS) was done on 29th day of the study. No difference in TBARS was observed in CP extract *per se,* SNEDDS *per se* when compared to vehicle group. Increase in the level of TBARS was observed in negative control group to which streptozotocin was administered, when compared to vehicle group as shown in table no. 6.25 and figure no. 6.9. This increase level of TBARS was reversed in the groups treated with low and high dose of CP extract and low and high dose of SNEDDS, as compared to disease control group. SNEDDS with high dose has shown the best result**.**

Diabetes and hyperglycaemia induce stress to the animals which reduces the level of reduced GSH in pancreas. Increase in level of GSH signifies less stress while decrease signify increased oxidative stress (Vaka et al., 2015; Simon et al., 2018). On the 29th day, GSH level was estimated as per method given by (Beutler *et al*., 1963). Highly significant difference was seen in negative control group in comparison to the normal control. The level of GSH in this group reduced due to stress of diabetes, significantly less difference in positive control was observed. No difference was observed in the GSH level of CP extract *per se,* SNEDDS *per se* group, when compared to vehicle group but decrease in the GSH level was observed in negative group when compared to vehicle group. This decrease effect of GSH was reversed in the groups treated with low and high dose of CP extract and low and high dose of SNEDDS which is comparatively better than positive control group. SNEDDS with high dose has shown the best result as shown in table no. 6.25 and figure no. 6.10.

Catalase activity study was done on 29th day of the experiment and no difference was observed in the CAT level in groups treated with CP extract *per se,* SNEDDS *per se*, when compared to vehicle group but decrease in the CAT level was observed in negative group when compared to vehicle group, as shown in table no. 6.25 and figure no. 6.11. This decrease effect of CAT was reversed in the groups treated with low and

high dose of CP extract and low and high dose of SNEDDS, as compare to disease control group. SNEDDS with high dose has shown the best result.

Chapter-7

Conclusion and Future perspectives

Current and traditional use of *Calotropis procera* was reviewed. The drug was authenticated and was subjected to analytical study. Physiochemical and phytochemical analysis was done along with safety evaluation parameters. The study was carried out to elucidate the anti-diabetic activity of the C.P extract. SNEDDS of C.P extract were characterised and optimised to overcome with the various drawbacks of plant-based formulations and compared with the extract. Labrafil, Tween 80, and tanscutol P were selected as oil, surfactant, and co surfactant (0.1 µl, 0.6 µl, and 0.3 µl) based on the solubility study. The characterized batch of SNEDDS shows 90.7% entrapment efficiency and particle was in the range of 50 nm as per TEM. Zeta potential lies in the range of-12.3 mv and PDI comes in the acceptable range i.e., 476. In dissolution study, SNEDDS allowed 99% dissolution whereas extract allowed 32% dissolution in 90 min. The dissolution rate of SNEDDS formulation was observed better and faster than extract, this is due to the fact of small droplet size of SNEDDS. SNEDDS were also subjected to the accelerated stability study. The accelerated study shows that the organoleptic characteristic i.e. description and odour were analysed for 3 months and 6 months. The optimised batch remain clear for the given period of time. Physio-chemical properties of optimised batch show no change in specific gravity, pH and viscosity for 6 months

Different concentrations of standard, SNEDDS and extract were used for *in-vitro* anti-diabetic and antioxidant activity and SNEDDS were found effective in comparison to standard and extract. *In-vivo* study investigated the effectiveness of low and high dose of CP extract and low and high dose of SNEDDS of CP extract in diabetes condition by estimating its effect on associated parameters (PGL, PTC and bodyweight). The results showed that CP extract *per se,* SNEDDS *per se* has no effect on the body weight of the healthy normal animals, but diabetic animals treated with low and high dose of extract and low and high dose of SNEDDS of extract has shown significant results in reducing the PGL and PTC level, when compared to disease control animal group. Changes in TBARS, GSH and CAT were also observed in the groups treated with low

and high dose of CP extract and low and high dose of SNEDDS of CP extract which showed its significant antioxidant potential. It can be concluded that *Calotropis procera* extract and SNEDDS of *Calotropis procera* extract is effective at both low and high doses. SNEDDS of extract with high dose has good efficacy in controlling PGL and PTC level. Therefore, SNEDDS prepared from *Calotropis procera* extract in high dose has good potential to be used for type 2 diabetes and can be further explored.

Chapter-8

Bibliography

1. Diagnosis and classification of diabeties mellitus, American diabeties association,Diabeties care 37(supplement), S81-S90,2104
2. Dr.Ambaddy Ramchandran, Indian diabeties research foundation, 2010;375:408-18
3. Tewari R C etal *Calotropis procerra*, Therapeutic and toxicological consideration.International Ayurvedic medical journal vol.3.2015.2384
4. P.Suresh etal, Review on potential herb calotropis, Scholors Academic journal of Pharmacy,2013;2(2):135-14
5. Ahmed KK, Rana AC, Dixit VK. Calotropis species (Ascelpediaceae): A comprehensive review. Pharmacognosy Magazine. 2005;1(2):48-52
6. Tewari R C etal. C.P. Therapeutic and toxicological consideration. International ayurvedic medical journal vol.3. 2015. 2384
7. Chaudhary P, Ahamad S, Khan N A. A review on medicinal utility of *calotropis procera*. World journal of pharmaceutical and medical research, 2017, 3 (1), 33
8. Sarvade Dattatray, D Bhingardive Kamini B , Jaiswal Mohanlal M. Arka (*calotropis procera*) a potent drug of Indian materia medica. World journal of pharmacy and pharmaceutical sciences vol 6, issue, 5, 471-487
9. Kumar VL, Padhy BM. Protective effect of aqueous suspension of dried latex of Calotropis procera against oxidative stress and renal damage in diabetic rats. Biocell. 2011;35(3):63–69
10. C.procera root extract has the capability to combat free mediated damage. International Scholarly Research Notices. 2013;1(2):1–8
11. Khairnar AK, Bhamare SR, Bhamare HP. *Calotropis procera*: An ethnopharmacological update. Advance Research in Pharmaceuticals and Biologicals. 2012;2(2):142–156.
12. Kumar S, Dewan S, Sangraula H, Kumar VL. Antidiarrhoeal activity of the latex of Calotropis procera. Journal of Ethnopharmacology. 2001;76(1):115–118.

13. Sharma A, Shahzad B, Kumar V, Kohli SK, Sidhu GPS, Bali AS, Zheng B. Phytohormones regulate accumulation of osmolytes under abiotic stress. Biomolecules. 2019;9(7):285
14. Mohsin K etal. Development of self-nanoemulsifying drug delivery systems for the enhancement of solubility and oral bioavailability of fenofibrate, a poorly water-soluble drug. International journal of nano medicine
15. Savale S.K. A review- Self nano emulsifying drug delivery system (SNEDDS) International Journal of research in Pharmaceutical and nano sciences (2015) 386
16. Gursoy RN, Benita S, self emulsifying drug delivery systems (SNEDDS) for improved oral delivery for lipophilic drugs. Biomed Pharmacother 2004; 58: 173-182.
17. Date A, Desai N, Nagarsenkar M. Self-nanoemulsifying drug delivery systems: Formulation insights, applications and advances. Future medicine ltd.2010; 1600
18. Pouton CW. Self emulsifying drug delivery system: accessment of efficiency of emulsification. Int J Pharm 1985; 27: 335- 338.
19. Gershanik T, Benita S. Self -dispersing lipid formulations for improving oral absorption of lipophilic drugs, Eur J Pharm Sci 2000; 50:179-188
20. 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.
21. Singh A etal. Self emulsifying systems: A review. Asian Journal of pharmaceutics 2015;15-16
22. Tiwari etal. Review on self emulsified drug delivery system: A primising approach for drug delivery of BCS class II and IV drugs
23. Bhanse N D, Shah C N. A Review of Research Study on-Self Nanoemulsifying Drug Delivery System (SNEDDS). Journal Pharma Science Bioscientific Res. 2016. 6(5):622
24. Kumar VL, Padhy BM, Sehgal R, Roy S. Antioxidant and protective effect of latex of Calotropis procera against alloxan-induced diabetes in rats. Journal of Ethnopharmacology. 2005;102(3):470–473.
25. The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed.1999, part I, Vol.-II,Apx-2 Page no190-191.

26. T. Prashant, Bimlesh Kumar et.al. Phytochemical screening and Extraction:A Review,International Pharmaceutical Science 2011;1: 98-106
27. Murti Y, Yogi B, Pathak D. Pharmacognostic standardisation of leaves of Calotropis procera R. Br. International journal of ayurveda research. 2010 Jan-Mar; 1 (1):14-17
28. Sarwadde Dattatray,D bhringardive, Kamini B, Jiaswal mohanlal M.Arka a potent drug of indian materia medica.World journal of pharmacy and pharmaceutical sciences vol 6, issue S5, 471-487
29. Reich E, Schibli A. Stationary Phases for Planar Separations - Plates for Modern TLC. LC GC. 2005;23:58–69
30. Dwivedi B etal. Evaluation of phytochemical constituents by gas chromatography-mass spectroscopy and HPTLC of *calotropis procera*. World Journal of pharmaceutical sciences vol.3 2017;711
31. The Ayurvedic Pharmacopoeia of India part 1 vol.1.First edition ;10,Ministry of health and family welfare.
32. Singh S, Kumar V, Chauhan A, Datta S, Wani AB, Singh N, Singh J. Toxicity, degradation and analysis of the herbicide atrazine. Environmental Chemistry Letters. 2018;16(1):211-237
33. Khairnar AK, Bhamare SR, Bhamare HP. Calotropis procera: An ethnopharmacological update. Advance Research in Pharmaceuticals and Biologicals. 2012;2(2):142–156.
34. Singh S, Singh N, Kumar V, Datta S, Wani AB, Singh D, Singh J. Toxicity, monitoring and biodegradation of the fungicide carbendazim. Environmental Chemistry Letters. 2016:14(3):317-329
35. Mathura R, Gupta SK. Anti-tumor studies with extracts of Calotropis procera (Ait.) R.Br. root employing Hep2 cells and their possible mechanism of action. Indian Journal of Experimental Biology. 2009;47: 343-48.
36. Bajaj H, Bisht, S, Yadav M, Singh V, Singh M.Self emulsifying drug delivery system: an approach to enhance Bioavailability. Int J Pharma 2011 Research, 3, 59-79.
37. Parmar N etal. Study of co-surfactant effect on nanoemulsifying area and development oflercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids and surfaces: Biointerfaces 86 (2011);328-329

38. Gupta S, Kesarla R, Omri A. Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems. International scholarly research notices 2013.
39. Kuruvila F, Mathew F, Kuppuswamy S. Solid self nano emulsifying drug delivery system Development, application, and future perspective: a review. Indo American Journal of pharmaceutical sciences 2017;4 (03)
40. Gershanik T, Benita S. Positively charged self-emulsifying oil formulation for improving oral bioavailability of progesterone. Pharmaceutical Development And Technology 1996;1:147-57
41. Felix Bornet..Thought about next generation (S) TEM instrument in science, micrin 2015; 90: 1-5 bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 2007;66:227-43
42. Pushpendra Kumar Jain, Navneet Khurana et.al., Enhancement of the absorption and hepato-protective potential- soya phosphatidylcholine andrographolide vesicular system, J Liposome Res,2013, 1532-2394
43. Shimamura etal. Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives. Interlaboratory evaluation study analytical science vol. 30, 2014;718
44. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-1008.
45. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. Food Science and Human Wellness. 2015;4(1):42-46.
46. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. Food Science and Human Wellness. 2015;4(1):42-46.
47. Narkhede M etal. In-vitro antidiabetic activity of caesalpinia digyana methanolic root extract. Asian journal of plant science and research.2011, 1(2) 105
48. Sah A, Raj S, Khatik G, Vyas M. Nutritional profile of spinach and its antioxidant and antidiabetic evaluation. Int J Green Pharm 2017;11:192-7

49. Mário C.L. Netoa, Carlos F.B. de Vasconcelosa. Evaluation of anti-hyperglycaemic activity of *Calotropis procera* leaves extract on streptozotocin-induced diabetes in Wistar rats. Brazilian Journal of Pharmacognosy.Rev Bras Farmacogn 23(2013): 913-919
50. Prabhakar PK, Prasad R, Ali S, Doble M Synergistic interaction of ferulic acid with commercial hypoglycemic drugs in streptozotocin induced diabetic rats. Phytomedicine. 2013;20(6):488-494.
51. Prabhakar PK, Prasad R, Ali S, Doble M. Synergistic interaction of ferulic acid with commercial hypoglycemic drugs in streptozotocin induced diabetic rats. Phytomedicine. 2013;20(6):488-494.
52. Kumar VL, Padhy BM, Sehgal R, Roy S. Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. Journal of Ethnopharmacology. 2005;102(3):470–473.
53. Murti Y, Yogi B, Pathak D. Pharmacognostic standardisation of leaves of *Calotropis procera* R. Br. International journal of ayurveda research. 2010 Jan-Mar; 1 (1):14-17
54. The ayurvedic pharmacopoeia of india part 1 vol.1.First edition ;10,Ministry of health and family welfare.
55. Dwivedi B etal. Evaluation of phytochemical constituents by gas chromatography-mass spectroscopy and HPTLC of *calotropis procera*. World Journal of pharmaceutical sciences vol.3 2017;711
56. Prosper T. Kinda1,6, Samson Guenné,Toxicological characterization and central nervous system effects of *Calotropis procera* aqueous extract in mice Asian Pacific Journal of Tropical Medicine 2019; 12(7): 329-336
57. Basu A, Chaudhari AK. Preliminary studies on the antiinflammatory and analgesic activities of *Calotropis procera* root extract. Journal of Ethnopharmacology. 1991;31(3):319–324.
58. Dewan S, Kumar S, Kumar VL. Antipyretic effect of latex of Calotropis procera. Indian Journal of Pharmacology. 2000;32(3):252- 253.
59. Vasconcelos SM. Antinociceptive activity of *Calotropis procera* latex in mice. Journal of Ethnopharmacology. 2005;99(1):125– 129.

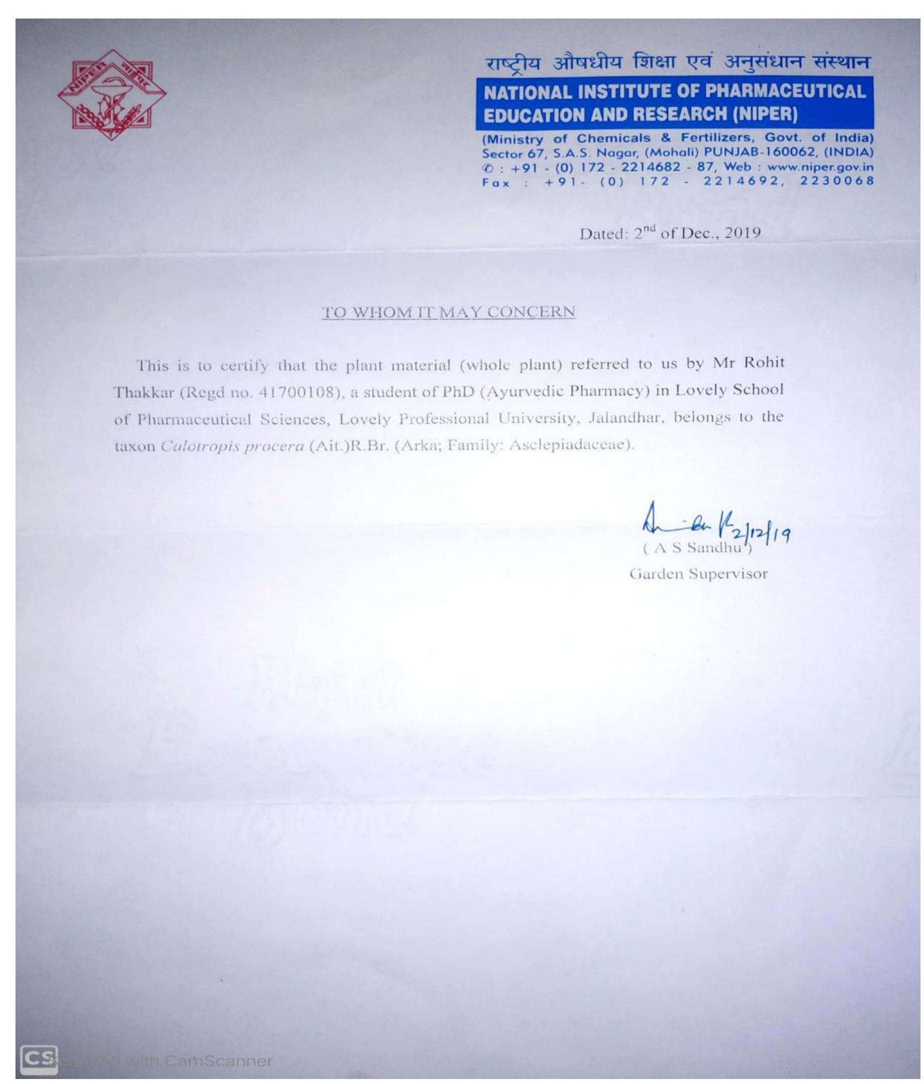
60. Sarwadde Dattatray,D bhringardive, Kamini B, Jiaswal mohanlal M.Arka a potent drug of indian materia medica.World journal of pharmacy and pharmaceutical sciences vol 6, issue S 5, 471-487
61. Aishwarya, Chopra V, Singh A, Vyas M Khurana N, Sharma N, Thakkar R, Singh H, Tandon N, Yadav P, Prajapati PK, Gupta M. A retrospective study on pharmacological activities of *Calatropis procera* Linn. Plant Cell Biotechnology and Molecular Biology. 2020;21(67-68):159-167
62. Prosper T. Kinda1,6, Samson Guenné,Toxicological characterization and central nervous system effects f *Calotropis procera*, its aqueous extract in miceAsian Pacific Journal of Tropical Medicine 2019; 12(7): 329-336
63. 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
64. Bhatia A, Singh B, Raza K, Wadhwa S, Katare OP. Tamoxifen-loaded lecithin organogel (LO) for the topical application: Development, optimization and characterization. International Journal of Pharmaceutics. 2013;444(1-2):47-59
65. Savale S.K. A review- Self nano emulsifying drug delivery system (SNEDDS) International Journal of research in Pharmaceutical and nano sciences (2015) 386
66. Gursoy RN, Benita S, self emulsifying drug delivery systems (SNEDDS) for improved oral delivery for lipophilic drugs. Biomed Pharmacother 2004; 58: 173-182.
67. Pouton CW. Self emulsifying drug delivery system: accessment of efficiency of emulsification. Int J Pharma 1985; 27: 335- 338
68. Parmar N etal. Study of cosurfactant effect on nanoemulsifying area and development oflercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system Colloids and surfaces b: Biointerfaces 86 (2011);328-329
69. Raza K, Thotakura N, Kumar P, Joshi M, Bhushan S, Bhatia A. Katare OP. C60-fullerenes for delivery of docetaxel to breast cancer cells: A promising approach for enhanced efficacy and better pharmacokinetic profile. International journal of pharmaceutics 2015;495(1):551-559.

70. Ujilestari T, Ariyadi B, Martien R, Zuprizal, Dono N. Optimization of self-nano emulsifying drug delivery systems of lemongrass (Cymbopogon citratus) essential oil. Int. J Appl Pharm 2019;11:144-9
71. Tiwari etal. Review on self emulsified drug delivery system: A primising approach for drug delivery of BCS class II and IV drugs
72. Kuruvila F, Mathew F, Kuppuswamy S. Solid self nano emulsifying drug delivery system Development, application, and future perspective: a review. Indo American Journal of pharmaceutical sciences 2017;4 (03)
73. Gupta S, Kesarla R, Omri A. Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems.International scholarly research notices 2013.
74. Pushpendra Kumar Jain, Navneet Khurana etal.., Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system, J Liposome Res,2013, 1532-2394
75. L, Zhang M, Pang Y, Li Z, Zhao A, et al. Selfemulsifying drug delivery system and the applications in herbal drugs. Drug Delivery 2015;22:475-86
76. Kumar D, Agarwal G, Tripathi B, Vyas D, Kulshrestha V. Characterization of PbS nanoparticles synthesized by chemical bath deposition. Journal of Alloys and Compounds. 2009;484(1-2):463-466.
77. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 2007;66:227-43
78. Patel,Archita, lalwani,Current drug delivery system,Vol.12,pp.745-760(16), Bentham science publisher.
79. Preethi G, Shivakumar H, Kumar M, Sweta N. Prototype self smulsifying system of Etravirine: Design, formulation and in vitro evaluation. Int J Appl Pharm 2018;10:13-9.
80. Saggu S, Sakeran MI, Zidan N, Tousson E, Mohan A, Rehman H. Ameliorating effect of chicory (Chichorium intybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food and Chemical Toxicology. 2014; 72:138-146.

81. Shoaib Quazi*, Kumkum Mathur, Sandeep Arora *Calotropis procera*: An overview of Its Phytochemistry and Pharmacology Indian Journal of Drugs, 2013, 1(2), 63-69 ISSN: 2348-1684
82. Quazi S, Mathur K, Arora S. *Calotropis procera.* An overview of its phytochemistry and pharmacological study. Indian Journal of Drugs. 2013;1(2):63-69.
83. Perwez A, Mohammad A. Phytochemical investigation of *Calotropis procera* roots, Indian Journal of Chemistry. 2009;48B(3): 443-446.
84. Verma R, Satsangi GP, Shrivastava JN. Ethno-medicinal profile of different plant parts of *Calotropis procera.* R.Br Ethnobotanical Leaflets. 2010;14:21-42
85. Quazi S, Mathur K, Arora S. *Calotropis procera*: An overview of its phytochemistry and pharmacology. Indian Journal of Drugs. 2013;1(2):63-69.
86. Basu A, Chaudhari AK. Preliminary studies on the antiinflammatory and analgesic activities of *Calotropis procera* root extract. Journal of Ethnopharmacology. 1991;31(3):319–324
87. Paul A, Kumar A. Review on pharmocological properties of Aaka (*Calotropis procera)*, International Journal of Economic Plants. 2018;5(3):157-162
88. Alzahrani, et al. Antitumor and antibacterial activity of *Calotropis procera* leaf extract. Journal of Basic and Applied Scientific Research. 2017;18.
89. P.suresh etal, Review on potential herb *C.procera*, **S**cholors Academic journal of Pharmacy,2013;2(2):135-143
90. Sarwadde Dattatray,D bhringardive, Kamini B, Jiaswal mohanlal M. Arka a potent drug of indian materia medica.World journal of pharmacy and pharmaceutical sciences vol 6, issue S 5, 471-487
91. *Calotropis procera*: An overview and Phytochemistry and Pharmacology Indian Journal of Drugs, 2013, 1(2), 63-69 ISSN: 2348-1684 Shoaib Quazi, Kumkum Mathur, Sandeep Aror

Appendices

Appendix-1



राष्ट्रीय औषधीय शिक्षा एवं अनुसंधान संस्थान

NATIONAL INSTITUTE OF PHARMACEUTICAL EDUCATION AND RESEARCH (NIPER)

(Ministry of Chemicals & Fertilizers, Govt. of India)
Sector 67, S.A.S. Nagar, (Mohali) PUNJAB-160062, (INDIA)
✆ : +91 - (0) 172 - 2214682 - 87, Web : www.niper.gov.in
Fax : +91- (0) 172 - 2214692, 2230068

Dated: 2nd of Dec., 2019

TO WHOM IT MAY CONCERN

This is to certify that the plant material (whole plant) referred to us by Mr Rohit Thakkar (Regd no. 41700108), a student of PhD (Ayurvedic Pharmacy) in Lovely School of Pharmaceutical Sciences, Lovely Professional University, Jalandhar, belongs to the taxon *Calotropis procera* (Ait.)R.Br. (Arka; Family: Asclepiadaceae).

2/12/19
(A S Sandhu)
Garden Supervisor

CS ... with CamScanner

Appendix -2

CIN No.- U24233PB2008PTC032243

Herbal Health Research Consortium Pvt. Ltd.

(A GOVT. OF INDIA SPONSORED & PB. GOVT. ASSISTED AYUSH CLUSTER PROJECT)

Works at : Village Khyala Khurd, Ram Tirth Road, Amritsar
Ph.: 01858-262025, Fax.: 01858- 262026 E-mail: herbheal@ymail.com
Corrs. Add: 277, East Mohan Nagar, Amritsar - 143006

Form No. 50 **A Government Approved Testing Laboratory**
Lic no. 1/ASU/TESTING LAB./PB./2014 **Dated: 20/10/2020**

CERTIFICATE OF ANALYSIS

(The Drugs and Cosmetics Act 1940 and the Rules Thereunder)

Sample	**AAK EXRACT** **(*Calotropis Procera*)**	**A.R. No.**	10/2020/TP/06
Supplied by	Rohit Thakkar	**Mfg. Date**	-
Date of receipt	07/10/2020	**Exp. Date**	-

Sr.No.	**TESTS**	**RESULTS**	**SPECIFIC REQUIREMENTS**
1.	**Microbial test**		
	a) Total Microbial Plate Count	40 cfu/g	1×10^5 cfu/g
	b) Total Yeast and Moulds	20 cfu/g	1×10^3 cfu/g
2.	**Pathogen Tests**		
	a) Escherichia coli	Absent	Absent
	b) Staphylococcus aureus	Absent	Absent
	c) Pseudomonas aeruginosa	Absent	Absent
	d) Salmonella	Absent	Absent
3.	**Heavy Metals (By ICP-OES)**		
	a) Lead (as Pb)	Not Detected	NMT 10.0 ppm
	b) Cadmium (as Cd)	Not Detected	NMT 0.3 ppm
	c) Mercury (as Hg)	Not Detected	NMT 1.0 ppm
	d) Arsenic (as As)	Not Detected	NMT 3.0 ppm
4.	**Test For Aflatoxins (B1, B2 and G1,G2)**	Not Detected	B1 & G1- NMT 0.5 ppm B2 & G2- NMT 0.1 ppm
5.	**HPTLC**	Complies	Q.S.I.M.P. Part 2,Page no 34 to 46

Note:

1. Sample(s) not drawn by us unless otherwise stated.
2. Total liability of this laboratory is limited to the invoiced amount.
3. Test certificate in full or parts shall not be used for promotional or publicity purpose.
4. The sample will be destroyed after one month of the date of issued test report, unless otherwise specified.

CIN No.- U24233PB2008PTC032243

Herbal Health Research Consortium Pvt. Ltd.

(A GOVT. OF INDIA SPONSORED & PB. GOVT. ASSISTED AYUSH CLUSTER PROJECT)
Works at : Village Khyala Khurd, Ram Tirth Road, Amritsar
Ph.: 01858-262025, Fax.: 01858- 262026 E-mail: herbheal@ymail.com
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Form No. 50 **Lic no. 1/ASU/TESTING LAB./PB./2014** **Dated: 20/10/2020**

CERTIFICATE OF ANALYSIS
(The Drugs and Cosmetics Act 1940 and the Rules Thereunder)

6.	Pesticide Residue(mg/Kg)	RESULTS	Pesticide Residue(mg/Kg)	RESULTS
	Endosulfan	Not detected	Dicholrvos	Not detected
	2-4' -DDT	Not detected	Malathion	Not detected
	Permethrin	Not detected	Parthion Ethyl	Not detected
	4-4' DDt	Not detected	Parthion Methyl	Not detected
	Gamma HCH	Not detected	2-4' DDE	Not detected
	Beta HCH	Not detected	2-4' DDD	Not detected
	Chlorpyrifos	Not detected	Alpha HCH	Not detected
	HeptaChlor	Not detected	Dieldrin	Not detected
	Aldrin	Not detected	Deltamethrin	Not detected
	4-4' DDD	Not detected	4--4' DDE	Not detected

Pooja Suman 20/10/2020 — **Prepared By**

20/10/2020 — **Checked By**

20/10/2020 — **Approved By**

"End of report"

qc_herbheal@ymail.com

Note:

1. Sample(s) not drawn by us unless otherwise stated.
2. Total liability of this laboratory is limited to the invoiced amount.
3. Test certificate in full or parts shall not be used for promotional or publicity purpose.
4. The sample will be destroyed after one month of the date of issued test report, unless otherwise specified.

Appendix-3

winCATS Planar Chromatography Manager

Herbal Health Research Consortium
Amritsar 143 001
Punjab

Analysis Report

Method	E:\2020\oct 2020\akk lpu student.cme	
Created by	Admin	Wednesday, October 14, 2020 12:04:50 AM
Last modified by	Admin	Wednesday, October 14, 2020 12:08:57 AM
SOP document		
Validated	Design	
Description :		

Analysis	E:\2020\oct 2020\akk method for scan plate aftersyray.cna	
Created/used by	Admin	Wednesday, October 14, 2020 2:19:26 AM
Current user	Admin	

Stationary phase

Executed by	Admin	Wednesday, October 14, 2020 12:09:40 AM
Plate size (X×Y)	5.0 x 10.0 cm	
Material		
Manufacturer		
Batch		
GLP code		
Pre-washing	No	
Modification	No	

Definitions - Quantification

Executed by	Admin	Wednesday, October 14, 2020 12:09:40 AM

Calibration parameters

Calibration mode	Single level
Statistics mode	CV
Evaluation mode	Peak height

Samples

Sample ID: akk sample 1

Sample ID: akk sample 2

Sample ID: akk sample 3

Sample ID: b-sitosterol std

Sample application - CAMAG Linomat 5

Instrument	CAMAG Linomat 5 "Linomat5_180745" S/N 180745 (1.00.12)	
Executed by	Admin	Wednesday, October 14, 2020 12:58:01 AM

Linomat 5 application parameters

Spray gas :	Inert gas
Sample solvent type :	Methanol
Dosage speed :	150 nl/s
Predosage volume :	0.2 ul

Sequence

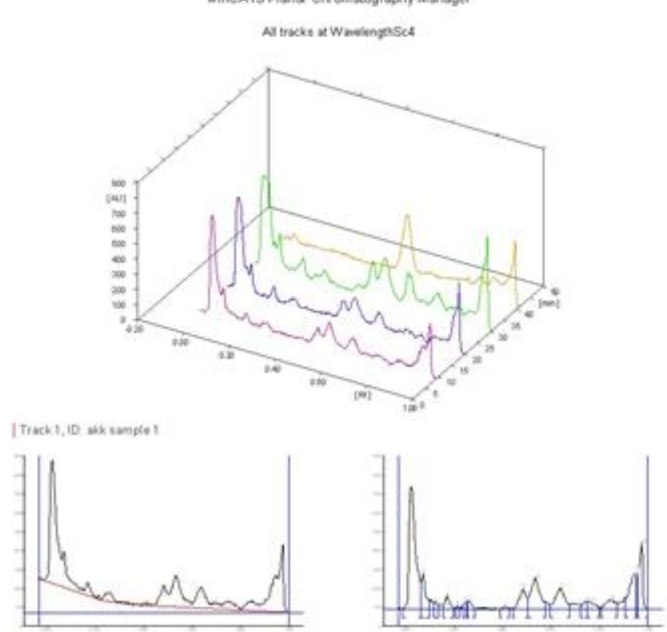
Syringe size:	100 µl
Number of tracks:	4
Application position Y:	8.0 mm
Band length :	6.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	9.0 mm	10.0 µl	1	akk sample 1	Yes

User : Admin
Wednesday, October 14, 2020 2:19:28 AM

Approved :. . . . .
Report ID : 07E40A0E0402131A

SN 1809W062, V1.4.6
Page 1 of 7

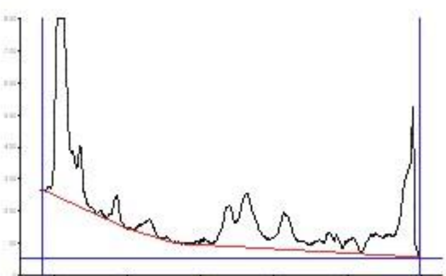


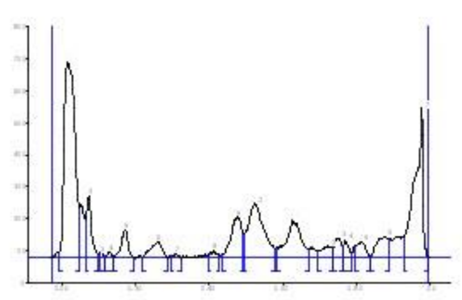
winCATS Planar Chromatography Manager
All tracks at WavelengthSc4
Track 1, ID: akk sample 1

winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.2	0.02	648.1	30.37	0.06	143.6	17994.4	33.05	unknown *
2	0.06	146.5	0.07	188.9	8.85	0.10	49.1	3093.8	5.68	unknown *
3	0.12	37.0	0.12	41.0	1.92	0.14	19.2	569.3	1.05	unknown *
4	0.15	21.1	0.17	67.2	3.15	0.20	9.9	1427.6	2.62	unknown *
5	0.21	5.4	0.21	19.2	0.90	0.22	9.7	181.9	0.33	unknown *
6	0.23	19.6	0.25	41.5	1.94	0.25	37.4	569.6	1.05	unknown *
7	0.25	37.5	0.26	46.4	2.17	0.29	2.3	846.2	1.55	unknown *
8	0.39	3.2	0.41	12.9	0.60	0.43	5.5	302.0	0.55	unknown *
9	0.44	1.7	0.48	111.5	5.23	0.50	63.3	3187.5	5.85	unknown *
10	0.50	63.4	0.53	165.7	7.77	0.58	38.9	6176.1	11.34	unknown *
11	0.60	23.9	0.64	110.8	5.19	0.68	23.5	4363.2	8.01	unknown *
12	0.72	22.0	0.74	44.6	2.09	0.75	38.2	903.6	1.66	unknown *
13	0.75	38.8	0.76	42.3	1.98	0.80	5.6	1202.9	2.21	unknown *
14	0.81	12.7	0.84	50.1	2.35	0.87	32.3	2132.6	3.92	unknown *
15	0.91	63.4	0.94	190.1	8.91	0.95	177.2	5508.0	10.12	unknown *
16	0.95	177.4	0.97	353.5	16.56	1.00	1.0	5984.4	10.99	unknown *

| Track 2, ID: akk sample 2





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	17.3	0.01	605.6	28.35	0.05	155.5	18096.2	31.28	unknown *
2	0.06	118.7	0.07	191.8	8.98	0.10	12.7	2864.9	4.95	unknown *
3	0.10	12.9	0.10	14.3	0.67	0.12	3.0	162.7	0.28	unknown *
4	0.12	3.6	0.13	19.2	0.90	0.14	4.9	207.6	0.36	unknown *
5	0.14	5.2	0.17	86.8	4.07	0.20	0.1	1628.3	2.81	unknown *
6	0.22	3.3	0.26	48.3	2.26	0.29	0.4	1556.7	2.69	unknown *
7	0.29	3.5	0.31	11.8	0.55	0.32	0.6	167.7	0.29	unknown *
8	0.40	6.9	0.41	22.4	1.05	0.43	9.0	377.3	0.65	unknown *
9	0.44	11.6	0.48	126.7	5.93	0.49	76.2	3797.9	6.57	unknown *
10	0.49	76.5	0.53	167.2	7.83	0.58	29.2	7412.4	12.81	unknown *
11	0.59	30.2	0.63	115.2	5.39	0.67	27.7	4665.0	8.06	unknown *
12	0.70	21.4	0.72	36.7	1.72	0.74	28.6	1075.8	1.86	unknown *
13	0.74	29.6	0.75	58.8	2.75	0.76	36.9	1182.7	2.04	unknown *
14	0.77	38.1	0.77	53.1	2.49	0.79	15.6	751.4	1.30	unknown *
15	0.80	35.1	0.81	49.2	2.30	0.84	6.9	1205.6	2.08	unknown *
16	0.84	6.9	0.88	65.5	3.07	0.89	57.5	2178.8	3.77	unknown *
17	0.93	63.0	0.98	463.5	21.70	1.00	1.8	10518.0	18.18	unknown *

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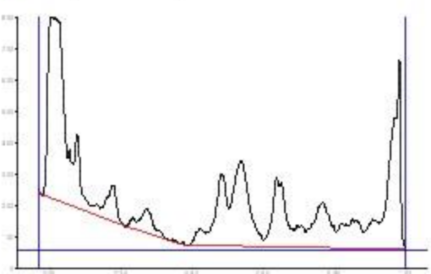
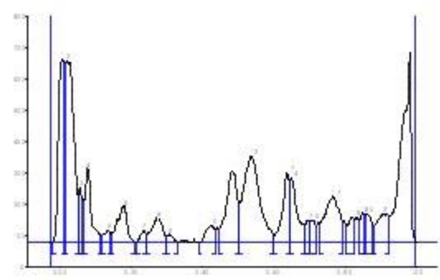
winCATS Planar Chromatography Manager

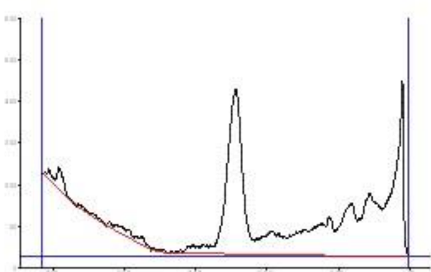
Track 3, ID: akk sample 3

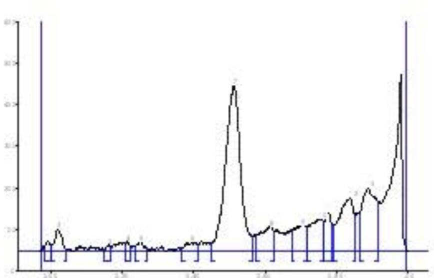




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.3	0.00	582.9	16.73	0.01	576.2	9736.5	11.86	unknown *
2	0.01	576.7	0.02	580.4	16.66	0.05	149.0	14499.8	17.67	unknown *
3	0.05	150.0	0.05	174.6	5.01	0.06	135.6	1715.8	2.09	unknown *
4	0.07	133.8	0.08	233.6	6.71	0.11	22.3	4204.1	5.12	unknown *
5	0.11	22.4	0.13	36.5	1.05	0.14	27.0	696.0	0.85	unknown *
6	0.14	27.3	0.18	115.0	3.30	0.21	0.6	3262.3	3.97	unknown *
7	0.21	1.6	0.23	35.8	1.03	0.24	26.1	567.8	0.69	unknown *
8	0.24	26.4	0.27	77.5	2.22	0.30	19.4	2400.9	2.93	unknown *
9	0.30	19.6	0.31	23.2	0.67	0.33	0.9	404.6	0.49	unknown *
10	0.39	0.1	0.42	54.1	1.55	0.43	40.8	1338.6	1.63	unknown *
11	0.44	42.5	0.48	229.4	6.58	0.50	122.5	7008.5	8.54	unknown *
12	0.50	122.6	0.54	274.0	7.86	0.60	22.6	12715.7	15.49	unknown *
13	0.60	23.2	0.64	220.8	6.34	0.64	195.2	4307.0	5.25	unknown *
14	0.64	197.7	0.65	208.1	5.97	0.68	55.9	4079.3	4.97	unknown *
15	0.69	56.3	0.69	69.9	2.01	0.70	65.1	897.2	1.09	unknown *
16	0.70	65.2	0.71	68.8	1.98	0.72	51.4	1010.5	1.23	unknown *
17	0.73	61.1	0.77	145.8	4.19	0.79	69.2	5941.5	7.24	unknown *
18	0.80	51.7	0.82	78.6	2.26	0.83	71.1	1434.2	1.75	unknown *
19	0.84	73.3	0.85	93.6	2.69	0.86	86.8	1190.6	1.45	unknown *
20	0.86	87.3	0.86	89.1	2.56	0.88	53.5	1553.5	1.89	unknown *
21	0.88	53.5	0.91	91.7	2.63	0.92	83.6	3106.3	3.78	unknown *

Track 4, ID: b-sitosterol std





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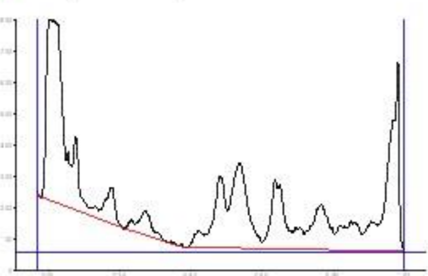
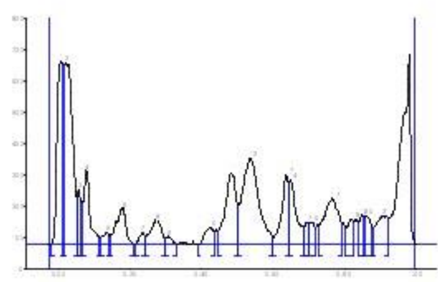
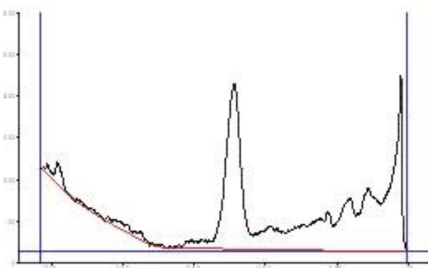
Track 3, ID: akk sample 3

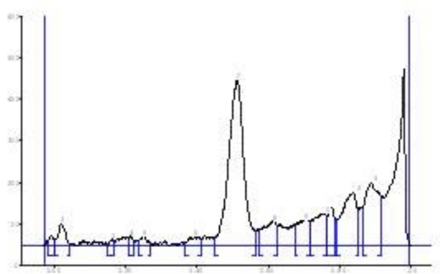




Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	0.3	0.00	582.9	16.73	0.01	576.2	9736.5	11.86	unknown *
2	0.01	576.7	0.02	580.4	16.66	0.05	149.0	14499.8	17.67	unknown *
3	0.05	150.0	0.05	174.6	5.01	0.06	135.6	1715.8	2.09	unknown *
4	0.07	133.8	0.08	233.6	6.71	0.11	22.3	4204.1	5.12	unknown *
5	0.11	22.4	0.13	36.5	1.05	0.14	27.0	696.0	0.85	unknown *
6	0.14	27.3	0.18	115.0	3.30	0.21	0.6	3262.3	3.97	unknown *
7	0.21	1.6	0.23	35.8	1.03	0.24	26.1	567.8	0.69	unknown *
8	0.24	26.4	0.27	77.5	2.22	0.30	19.4	2400.9	2.93	unknown *
9	0.30	19.6	0.31	23.2	0.67	0.33	0.9	404.6	0.49	unknown *
10	0.39	0.1	0.42	54.1	1.55	0.43	40.8	1338.6	1.63	unknown *
11	0.44	42.5	0.48	229.4	6.58	0.50	122.5	7008.5	8.54	unknown *
12	0.50	122.6	0.54	274.0	7.86	0.60	22.6	12715.7	15.49	unknown *
13	0.60	23.2	0.64	220.8	6.34	0.64	195.2	4307.0	5.25	unknown *
14	0.64	197.7	0.65	208.1	5.97	0.68	55.9	4079.3	4.97	unknown *
15	0.69	56.3	0.69	69.9	2.01	0.70	65.1	897.2	1.09	unknown *
16	0.70	65.2	0.71	68.8	1.98	0.72	51.4	1010.5	1.23	unknown *
17	0.73	61.1	0.77	145.8	4.19	0.79	69.2	5941.5	7.24	unknown *
18	0.80	51.7	0.82	78.6	2.26	0.83	71.1	1434.2	1.75	unknown *
19	0.84	73.3	0.85	93.6	2.69	0.86	86.8	1190.6	1.45	unknown *
20	0.86	87.3	0.86	89.1	2.56	0.88	53.5	1553.5	1.89	unknown *
21	0.88	53.5	0.91	91.7	2.63	0.92	83.6	3106.3	3.78	unknown *

Track 4, ID: b-sitosterol std





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Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	8.1	-0.01	24.1	2.18	0.00	13.2	310.1	0.78	unknown *
2	0.00	13.3	0.02	50.5	4.58	0.04	2.7	995.8	2.51	unknown *
3	0.15	0.6	0.16	13.7	1.24	0.17	8.5	139.0	0.35	unknown *
4	0.21	15.3	0.21	19.1	1.74	0.22	8.7	250.4	0.63	unknown *
5	0.23	12.0	0.25	21.3	1.93	0.27	4.5	410.6	1.03	unknown *
6	0.37	6.5	0.39	18.6	1.69	0.41	15.3	591.5	1.49	unknown *
7	0.46	17.5	0.51	394.8	35.78	0.57	34.4	16832.3	42.39	unknown *
8	0.57	36.3	0.62	56.3	5.10	0.63	47.7	2167.3	5.46	unknown *
9	0.67	48.6	0.70	60.7	5.50	0.72	57.4	2133.0	5.37	unknown *
10	0.72	57.8	0.75	76.7	6.96	0.77	69.5	2878.2	7.25	unknown *
11	0.77	69.6	0.77	91.4	8.29	0.79	60.0	1659.1	4.18	unknown *
12	0.79	61.4	0.84	126.7	11.48	0.86	86.1	5342.7	13.45	unknown *
13	0.87	89.2	0.89	149.4	13.54	0.92	116.8	6001.1	15.11	unknown *

Spectrum scan

Executed by	Admin Wednesday, October 14, 2020 2:17:38 AM
Mode	All detected peaks
Slit dimensions	6.00 x 0.30 mm, Micro
Optimize optical system	Resolution
Scanning speed	100 nm/s
Data resolution	1 nm/step
Reference spectrum, pos X	10.0 mm
Reference spectrum, pos Y	10.0 mm

Measurement Table

Lamp	D2 & W
Start wavelength	450 nm
End wavelength	550 nm
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector Mode	Automatic

Detector properties

Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (67)

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	akk sample 1
2	Sample	2	akk sample 2
3	Sample	3	akk sample 3
4	Sample	4	b-sitosterol std

Table of substances

Substance	Position MD mm	Tracks 1 2 3 4

Results per track

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Report ID : 07E40A0E0402131A

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winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	8.1	-0.01	24.1	2.18	0.00	13.2	310.1	0.78	unknown *
2	0.00	13.3	0.02	50.5	4.58	0.04	2.7	995.8	2.51	unknown *
3	0.15	0.6	0.16	13.7	1.24	0.17	8.5	139.0	0.35	unknown *
4	0.21	15.3	0.21	19.1	1.74	0.22	8.7	250.4	0.63	unknown *
5	0.23	12.0	0.25	21.3	1.93	0.27	4.5	410.6	1.03	unknown *
6	0.37	6.5	0.39	18.6	1.69	0.41	15.3	591.5	1.49	unknown *
7	0.45	17.5	0.51	394.8	35.78	0.57	34.4	16832.3	42.39	unknown *
8	0.57	36.3	0.62	56.3	5.10	0.63	47.7	2167.3	5.46	unknown *
9	0.67	48.6	0.70	60.7	5.50	0.72	57.4	2133.0	5.37	unknown *
10	0.72	57.8	0.75	76.7	6.96	0.77	69.5	2878.2	7.25	unknown *
11	0.77	69.6	0.77	91.4	8.29	0.79	60.0	1659.1	4.18	unknown *
12	0.79	61.4	0.84	126.7	11.48	0.86	86.1	5342.7	13.45	unknown *
13	0.87	89.2	0.89	149.4	13.54	0.92	116.8	6001.1	15.11	unknown *

Spectrum scan

Executed by	Admin Wednesday, October 14, 2020 2:17:38 AM
Mode	All detected peaks
Slit dimensions	6.00 x 0.30 mm, Micro
Optimize optical system	Resolution
Scanning speed	100 nm/s
Data resolution	1 nm/step
Reference spectrum, pos X	10.0 mm
Reference spectrum, pos Y	10.0 mm

Measurement Table

Lamp	D2 & W
Start wavelength	450 nm
End wavelength	550 nm
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector Mode	Automatic

Detector properties

Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic(67)

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	akk sample 1
2	Sample	2	akk sample 2
3	Sample	3	akk sample 3
4	Sample	4	b-sitosterol std

Table of substances

Substance	Position MD mm	Tracks 1 2 3 4

Results per track

Appendix -4

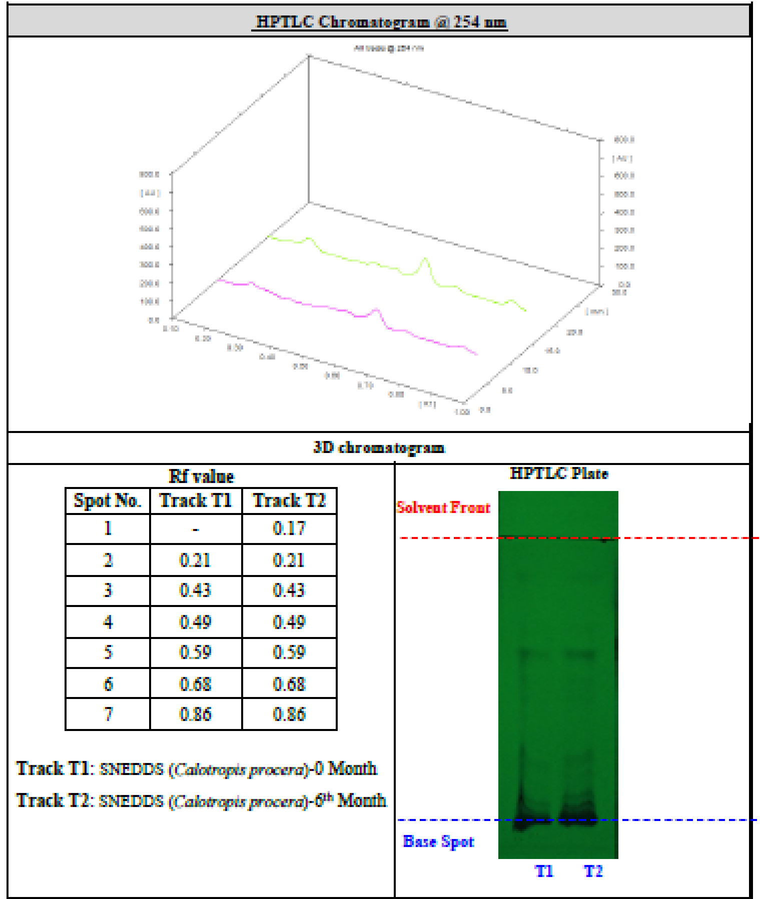
SNEDDS (*Calotropis procera*)		Report Date	01.02.2022
		Sample ID	AD/21/262
Name of Scholar	Dr. Rohit Thakkar, PG Scholar, Lovely Professional University, Punjab		

ACCELERATED STABILITY STUDY (TEMP: 40±2 °C, 75±5 % RH)				
Sr. No.	Parameters	0 Month	3rd Month	6th Month
ORGANOLEPTIC ANALYSIS				
1	Description	Milky white liquid	Milky white liquid	Milky white liquid
2	Odour	Characteristic	Characteristic	Characteristic
PHYSICO-CHEMICAL ANALYSIS				
1	Specific gravity	1.017	1.021	1.025
2	pH	3.31	3.32	3.35
3	Viscosity	0.62 cP	0.64 cP	0.68 cP
INSTRUMENTAL ANALYSIS				
1	HPTLC Fingerprinting	Report attached	Not Applicable	Report attached
MICROBIAL ANALYSIS				
1	Total Microbial Plate Count	< 10 cfu/g	Not Applicable	< 10 cfu/g
2	Total Yeast & Mould Count	Absent		Absent
3	*Staphylococcus aureus*	Absent		Absent
4	*Salmonella sp.*	Absent		Absent
5	*Pseudomonas aeruginosa*	Absent		Absent
6	*Escherichia coli*	Absent		Absent
Key Word: ppm - Parts per millions; ND - Not detected; cfu/g – Colony forming unit per gram				

Appendix-5

HPTLC FINGERPRINTING REPORT		
Sample	:	SNEDDS (*Calotropis procera*)
Name of Scholar	:	Dr. Rohit Thakkar, PG Scholar, Lovely Professional University, Punjab
Sample ID	:	AD/21/262
Date of Report	:	01.02.2022
Preparation of Test solution: Sample was evaporated and residue was dissolved in 1 mL methanol. Collected solution was then filtered with 0.45 µm membrane filter. Obtained test solution was then used for HPTLC fingerprinting.		
Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]: 0.5 mL Anisaldehyde is mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98 %).		
Chromatographic Conditions:		

Parameter	Condition
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	25.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl acetate : Methanol (8 : 2 : 0.5 v/v)
Visualization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Spray reagent	Anisaldehyde Sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 5^0C for 3 minutes



HPTLC Chromatogram @ 254 nm

3D chromatogram

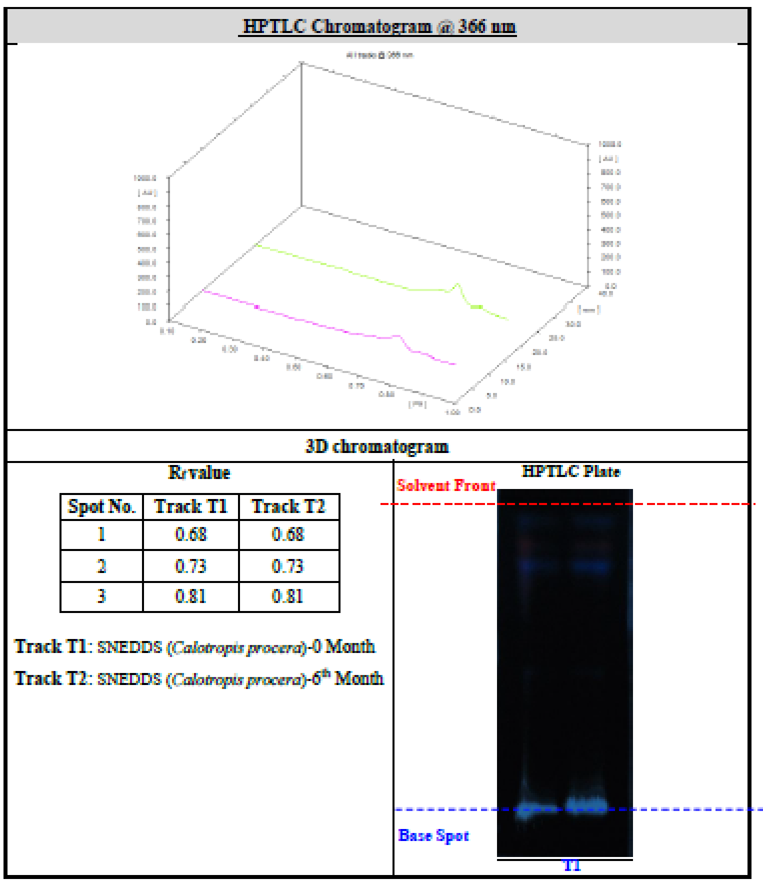
Rf value

Spot No.	Track T1	Track T2
1	-	0.17
2	0.21	0.21
3	0.43	0.43
4	0.49	0.49
5	0.59	0.59
6	0.68	0.68
7	0.86	0.86

Track T1: SNEDDS (*Calotropis procera*)-0 Month

Track T2: SNEDDS (*Calotropis procera*)-6th Month

HPTLC Plate



HPTLC Chromatogram @ 366 nm

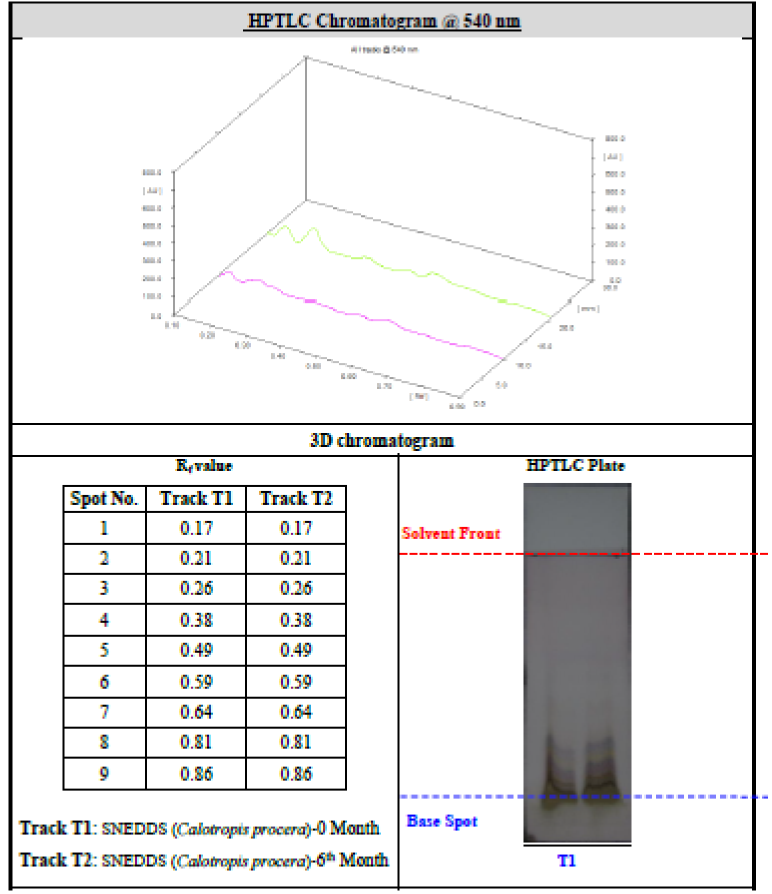
3D chromatogram

R_f value

Spot No.	Track T1	Track T2
1	0.68	0.68
2	0.73	0.73
3	0.81	0.81

Track T1: SNEDDS (*Calotropis procera*)-0 Month

Track T2: SNEDDS (*Calotropis procera*)-6th Month



HPTLC Chromatogram @ 540 nm

3D chromatogram

R_f value

Spot No.	Track T1	Track T2
1	0.17	0.17
2	0.21	0.21
3	0.26	0.26
4	0.38	0.38
5	0.49	0.49
6	0.59	0.59
7	0.64	0.64
8	0.81	0.81
9	0.86	0.86

Track T1: SNEDDS (*Calotropis procera*)-0 Month

Track T2: SNEDDS (*Calotropis procera*)-6th Month

HPTLC Plate

List of Publications

1) Review article entitled **“**A Retrospective study on Pharmacological Activities of Calotropis procera Linn.” published in Plant Cell Biotechnology and Molecular Biology 21(67&68):159-167; 2020.
2) Research article entitled “*In-vitro* Evaluation of Anti-Oxidant and Anti-Diabetic Activity of *Calotropis procera”* Published in Plant Cell Biotechnology and Molecular Biology. 22(33&34):288-292; 2021.
3) Paticipated in International Conference on “Recent Advances in Fundamental and Applied Sciences” (RAFAS-2021)
4) Participated in “International conference of Material for Emerging Technologies” (ICMET-21)

Plant Cell Biotechnology and Molecular Biology 22(33&34):288-292; 2021 ISSN: 0972-2025

In-vitro EVALUATION OF ANTI-OXIDANT AND ANTI-DIABETIC ACTIVITY OF *Calotropis procera*

ROHIT THAKKAR, RAJAN KUMAR, AMRIT PAL SINGH, MANISH VYAS*, NAVNEET KHURANA, NEHA SHARMA, DHARAM BUDDHI, PRAMOD YADAV, P. K. PRAJAPATI AND BHUPENDRA TOMAR

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Article Information

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Original Research Article

ABSTRACT

Introduction: Herbal plants are known for their anti-oxidant and other various therapeutic activities. Mostly the active chemical moieties of the herbal plants are flavonoids in nature. In the present study, Calotropis procera was evaluated for its anti-oxidant and anti-diabetic activity.

Materials and Methods: First the ethanolic extract of leaves of the plant was prepared and then subjected to the in-vitro anti-oxidant and anti-diabetic evaluation. Anti-oxidant activity was evaluated by using DPPH and hydrogen peroxide assay whereas Alpha amylase assay was performed to evaluate the anti-diabetic activity. The result of the extract was also compared with the standard compound.

Results and Discussion: The IC_{50} value for extract was found to be less when compared with the standard which represents that extract have good anti-oxidant and anti-diabetic activity.

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A RETROSPECTIVE STUDY ON PHARMACOLOGICAL ACTIVITIES OF *Calatropis procera* LINN

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Review Article

ABSTRACT

Plants and herbs are being used for the treatment of various diseases since ancient time due to their efficacy and availability. According to the World Health Organization, 80 % world population use herbs or medicinal plants in one or the other way as vegetables, spices, and medicines. These drugs can be used internally and externally to treat various disorders, which are also reported in recent studies. However, there is a need to explore their therapeutic potential by conducting pre-clinical and clinical studies. Numerous plants have been used in human life for various purposes, one such plant is *Calatropis procera* (*C. procera*). It has been used since ancient times and also reported to have multidimensional therapeutic activities in Ayurvedic, Arabic, and Unani medicine system. It consists of a variety of chemical constituents including stigmasterol, β-sitosterol, flavonoids, polyphenols, calotropin, calotropagenin, and calotoxin. Recent studies suggest its role in the management of bronchitis, baldness, hair loss, asthma, leprosy, intermittent fevers, eczema, elephantiasis, vertigo, toothache,

Conference-1

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Certificate of Participation

This is to certify that **Mr. Rohit Thakkar**

of **Lovely Professional University**

has given poster presentation on **Quality Assessment of Leaves of Calotropis procera Linn. with its In-Vitro Antioxidant and Antidiabetic Evaluation**

in the International Conference on "Recent Advances in Fundamental and Applied Sciences" (RAFAS 2021) held on June 25-26, 2021, organized by School of Chemical Engineering and Physical Sciences, Lovely Faculty of Technology and Sciences, Lovely Professional University, Punjab.

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Certificate of Participation

This is to certify that **Mr. Rohit Thakkar** of **Lovely Professional University, Phagwara, Punjab, India** has presented paper on **Formulation Development and In vitro antioxidant and antidiabetic evaluation of calotropis procera based SNEDDS** in the **International Conference on Materials for Emerging Technologies (ICMET-21)** held on February 18-19, 2022, organized by Department of Research Impact and Outcome, Division of Research and Development, Lovely Professional University, Punjab.

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CERTIFICATE

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Rohit Thakkar

has attended National Level webinar on topic ***"Immunomodulation through Ayurveda"*** delivered by Dr Manish Vyas, Associate Professor, School of Pharmaceutical Sciences, Lovely Professional University Phagwara, Punjab On 29/05/2021, conducted by B. R. Nahata College of Pharmacy, Faculty of Pharmacy, under IQAC Cell, Mandsaur University, Mandsaur (M.P.)

Mr. Sanjay Jain	Dr. M. A. Naidu	Dr. Amit K Jain
Co-ordinator	Co-ordinator	Co-ordinator-IQAC

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Certificate No.240558

Certificate of Recognition

This is to certify that **Rohit Thakkar** of **Lovely Professional University, Phagwara, Punjab, India** has contributed as **Student Coordinator** in the **International Conference on Materials for Emerging Technologies (ICMET-21)** held on February 18-19, 2022, organized by Department of Research Impact and Outcome, Division of Research and Development, Lovely Professional University, Punjab.

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Workshop-3

Certificate No.246125

Certificate of Participation

This is to certify that **Mr. Rohit** of **Lovely Professional University** has participated in the "**Awareness Program to Introduce Giloy in Routine Life for The Promotion of Health**" held on **26th March 2022**, organized under the funded project entitled ***"National Campaign on Amrita for Life (Tinospora cordifolia)"***at Lovely Professional University, Punjab.

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