DEVELOPMENT OF VESICULAR DRUG DELIVERY SYSTEM FOR WITHANIA SOMNIFERA

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Submitted By

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In partial fulfilment for the award of the degree of

MASTER OF PHARMACY IN

PHARMACEUTICS

Under the Guidance Of

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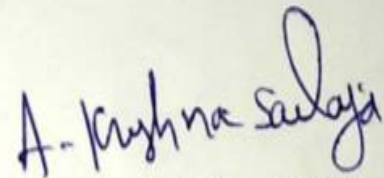

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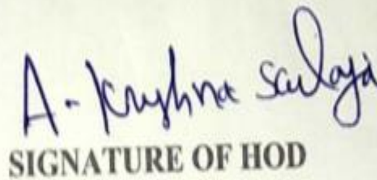

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DEDICATED TO MY

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CONTENT

List Of Tables

List Of Figures

List Of Abbrevations

Abstract

Chapter-1

Introduction 1-16

1.2 .Withania Somnifera

1.3 Components Of Withania Somnifera

1.3 Applications Of Withania Somnifera

1.4 Introduction Of Ethosomes

1.4.2 Advantages Of Ethosomal Drug Delivery

1.4.3 Disadvantages Of Ethosomal Drug Delivery

1.4.4 Mechanism Of Drug Penetration

1.5 Methods Of Preparation Of Ethosomes

1. Cold Method

2. Hot Method

1.5.1 Characterization Of Ethosomes

Therapeutics Application Of Ethosomes

Chapter-2 17-31

2. Review Of Literature

2.1. General Work

2.2. Conclusion Drawn From Literature Review

2.3. Need Of The Study

2.4. Aim And Objective Of The Study

Chapter-3 32-51

3. Methodology

3.1 Materials Used

3.3 Plant Profile

3.4 Discription Of Withania Somnifera

3.7 Excipient Profile

3.8 Experimental Methodology

3.9 Preliminary Phytochemical Studies Of Root Extract Of Withania Somnifera:

3.10 Antimicrobial Activity Of Extracts

3.11 Thin Layer Chromatography (Tlc)

3.12 Preparation Of Ethosomes From The Root Extract Of Withania Somnifera By Cold And Hot Method

3.15 Characterization And Evaluation Of The Ethosomes

3.16 Evaluation Of Ethosomes

Chapter-4 52-76

4.1 Extraction:

4.3 Thin Layer Chromatography

4.4 Antimicrobial Activity

4.5 Evaluation Parameters

4.6 Results And Discussion Of Ethosomal Formulation Of Withania Somnifera By Cold Method

4.7 Results And Discussion Of Ethosomal Formulation Of Withania Somnifera By Hot Method

4.8 Comparative Study Among The Best Formulations Of Withania Somnifera Loaded Ethosomes By Hot And Cold Method

Chapter-5 79-80

Conclusion

Chapter-6 81-88

Reference

Annaxure-1 90-93

Annaxure-2 94-98

LIST OF TABLES

Table No	Name of Table	Page No
1.4.1	Different additives employed in formulation of ethosomes	10
1.5.2	Marketed Products Based On Ethosomal Drug Delivery System	15
3.1	list of all chemicals used	33
3.2	List of all equipment's used	34
3.5	Vernacular names of withania somnifera	36
3.6	Pharmacognosy of withania somnifera	36
3.13	List and composition of formulations prepared by Cold method	47
3.14	List and composition of formulations prepared by Hot method	49
4.2	Determination Of Phytochemical Constituents Of Withania Somnifera.	53
4.9	Invitro drug release data of E6 best formulation of withania somnifera loaded ethosomes by cold method.	75
4.9.2	Kinetic data of E6 formulation of ethosomes by cold method.	77

LIST OF FIGURES

Figure No	Name of the figure	Page No
1.1	Structure Of Ethosomes	9
1.3.4	Mechanism Of Ethosomes	12
3.6	Withania Somnifera	37
3.6.1	Roots Of Withania Somnifera	37
3.12.1	Cold Method	47
4.1	Dried Withania Somnifera Root Extract	52
4.3	Tlc Study Of Root Extract Of Withania Somnifera	53

4.4.1	Anti-Microbial Activity Of Withania Somnifera Against Staphylococcus	56
4.4.2	Anti-Microbial Activity Of Withania Somnifera Against Pseudomonas.	56
4.4.3	Anti-Microbial Activity Of Withania Somnifera Against Ecoli.	56
4.6.1	Projection Microscopic Images Of Ethosomes Prepared By Cold Method	58
4.6.2	Comparison Of Mean Vesicle Diameter Of Withania Somnifera Loaded Ethosomes By Cold Method	59
4.6.3	Vesicle Size Distribution Report Of Optimized E6 Formulation Of Withania Somnifera Loaded Ethosomes By Cold Method.	60
4.6.4	Comparison Of Zeta Potential Values Of Six Formulations Of Withania Somnifera Loaded Ethosomes By Cold Method.	61
4.6.5	Zeta Potential Report Of Optimized E6 Formulation Of Withania Somnifera Loaded Ethosomes By Cold Method.	62
4.6.6	Comparison Of Drug Content Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Cold Method.	63
4.6.7	Comparison Of Entrapment Efficiency Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Cold Method.	63

4.6.8	Comparison Of Invitro Drug Diffusion Studies Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Cold Method.	64
4.7.1	Projection Microscopic Images Of Ethosomes Prepared By Hot Method	66
4.7.2	Comparison Of Mean Vesicle Diameter Of Withania Somnifera Loaded Ethosomes By Hot Method.	66
4.7.3	Vesicle Size Distribution Report Of Optimized E9 Formulation Of Withania Somnifera Loaded Ethosomes By Hot Method.	66
4.7.4	Comparison Of Zeta Potential Of Six Formulations Of Withania Somnifera Loaded Ethosomes By Hot Method.	68
4.7.6	Comparison Of Drug Content Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Hot Method.	70
4.7.7	Comparison Of Entrapment Efficiency Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Hot Method.	70
4.7.8	Comparison Of Invitro Drug Diffusion Studies Among The Six Formulations Of Withania Somnifera Loaded Ethosomes By Hot Method.	71

4.8.1	Vesicle Size Diameter Of Optimized Ethosomal Formulations	72
4.8.2	Zeta Potential Values Of Optimized Ethosomal Formulations	73
4.8.3	Drug Content Of Optimized Ethosomal Formulations	73
4.8.4	Entrapment Efficiency Of Optimized Ethosomal Formulations	74
4.8.5	% Drug Release Profile Of Optimized Ethosomal Formulations	74
4.9.1	Comparison Of Optimized Ethosomal Formulation E6 By Cold Method With Various Kinetic Models.	77

ABBREVIATIONS

WS	withania somnifera
%	Percentage
EE	Entrapment efficiency
DC	Drug content
UV	Ultra violet spectroscopy
RBF	Round bottomed flask
Gm	Gram
Hr	Hour
Min	Minutes
RPM	Revolutions per minute
R^2	Correlation coefficient
N	Release exponent
Mg	Milligram
Sec	Seconds
Mg/ml	Milligrams per milliliter
μg/ml	Micrograms per milliliter
SEM	Scanning electron microscope
%w/v	Percentage weight by volume
%v/v	Percentage volume by volume
Log T	Logarithm of time
Cum	Cummulative
$T^{1/2}$	Square root of time
AM	Antimicrobial
AC	Anticancer
TLC	Thin layer chromatography
PCS	Photon correlation spectroscopy
CLSM	confocal laser scanning microscopy
SEM	Scanning electron microscopy

ABSTRACT

The aim of the present study is to develop Ethosomal formulation of withania somnifera using cold and hot methods . Withania Somnifera (solanaceae), also known as Ashwagandha or winter cherry, is one of the most valuable plants in the traditional Indian systems of medicine. The plant is known to possess anti-inflammatory, antitumor, antistress, antioxidant and immunomodulatory properties. Eventhough Withania somnifera possess these properties but the basic problem is poor bioavailability. So, there is a need to develop Withania somnifera into novel vesicular drug delivery system i.e. ethosomal drug delivery system which results in particle size reduction and will enhances the penetration through skin and also increases bioavailability. The dried root extraction of withania somnifera was obtained using soxhlet apparatus. The phytochemical screening tests was revealed the presence of Tannins, Alkaloids, Steroids, Flavanoids, Saponins, Ammino acids and proteins. TLC revealed the ethanolic root extract of withania somnifera contains steroids. Rf values was found to be 0.88 for the standard withanolide and 0.99 for the root extract of withania somnifera indicate the presence of withanolide. Attempts have been made to prepare and evaluate Withania somnifera loaded ethosomes. Total twelve formulations (E1-E12) of ethosomes were prepared by using hot and cold method by using soya lecithin as lipid, ethanol, propylene glycol as solvents. First six formulations were prepared by cold method by varying drug to lipid ratio. Other six formulations were prepared by hot method by varying drug to lipid ratio. The prepared ethosomal formulations were evaluated for drug content, entrapment efficiency, invitro drug release studies, zeta potential and mean particle size. Among the twelve formulations of ethosomes E6 of drug: lipid (1:6) ratio was found to be the best formulation with drug content of 98%, entrapment efficiency of 95.6%, zeta potential of -20.0 mV, mean particle diameter of 254.6nm and invitro drug release of 98% in a time period of 12hrs. In this present study Withania somnifera loaded ethosomes were successfully prepared and evaluated.

Keywords: Ethosomes, Withania somnifera, Cold method and hot method.

CHAPTER -1
INTRODUCTION

1. INTRODUCTION

1.1 WITHANIA SOMNIFERA

Withania Somnifera (solanaceae), also known as Ashwagandha or winter cherry, is one of the most valuable plants in the traditional Indian system of medicine. It is a small evergreen shrub that grows roughly four to five feet tall. In India, it is cultivated, on a commercial scale, in the states of Madhya pradesh, Uttar pradesh, Punjab, Gujarat and Rajasthan. This plant is used in more than 100 formulations in ayurveda, unani and siddha(1).

Ashwagandha is one of the prime drugs of ayurveda material medica. It is attributed with balya, vrishya and rasayana properties and it is a substitute of kakoli and kshirakakoli(2). Withania somnifera has been an important herb in the ayurvedic and indigenous medical systems for over 3000 years(3).

Ashwagandha in Sanskrit means "horse's smell" probably originated from the odour of its root. The species name somnifera means "sleep-making" in Latin(4), attributed to sedating properties, it has been used for sexual vitality and as adaptogenic properties also. As a rasayana herb, the decoction and extracts of the herb shows excellent immunomodulatory activity by non-specific activation of macrophages, granulocytes, complement systems, natural killer cells and lymphocytes(2). The plant is known to possess anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory and hemopoetic properties.

Various withanolides, steroidal lactones, have been isolated from *W.somnifera* and were known to have high therapeutic value. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, antiinflammatory agent, astringent and recently it is used to treat ulcers, bacterial infection, venom toxins and senile dementia(5). Clinical trials and animal research support the use of WS for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson’s diseases. Recently WS is also used to inhibit the development of tolerance and dependence on chronic use of various psychotropic drugs(6). *Withania* species show a particularly wide distribution throughout climates of the world.

Chemical composition:

The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds[7], including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27. Apart from these contents plant also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane and ducitol, a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron. The biologically active chemical constituents of Withania somnifera (WS) include alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins. Sitoindosides and acylsterylglucosides in Ashwagandha are anti-stress agents[8].Active principles of Ashwagandha, for instance the sitoindosides VII-X and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress. Many of its constituents support immunomodulatory actions. The aerial parts of Withania somnifera yielded 5-dehydroxy withanolide-R and withasomniferin-A.

1.2 Components of withania somnifera:

- Alkaloids
- Tannins
- Carbohydrates
- Steroids
- Saponins
- Flavonoids

1.3 APPLICATIONS OF WITHANIA SOMNIFERA:

- The root of ashwagandha is used as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. The root smells like horse ("*ashwa*"), that is why it is called Ashwagandha. It is commonly used in emaciation of children debility from old age, rheumatism, constipation, insomnia, nervous breakdown, goiter etc[9]. The paste which

was prepared from roots of withania somnifera is used to reduce the inflammation at the joints. It is also locally applied in carbuncles, ulcers and painful swellings. The root in combination with other drugs is prescribed for snake venom as well as in scorpion-sting. It also helps in leucorrhoea, boils, pimples, flatulent colic, worms and piles. The Nagori Ashwagandha is the supreme among all ashwagandha varieties. Maximum benefit appears when fresh ashwagandha root powder is used.

- The leaves are used to treat fever, painful swellings. The flowers are used as astringent, depurative, diuretic and aphrodisiac(10) . The seeds are used to remove white spots from the cornea and used in hysteria, anxiety, memory loss, syncope, etc. It also acts as a stimulant and increases the sperm count.

Anti-oxidant Effects of Ashwagandha:

Free radical scavenging activity of root powder of ashwagandha in mice is observed that root powder possesses free radical scavenging activity, which is responsible for its pharmacological effects(11). The active principles of Ashwagandha, sitoindosides VII-X and withaferine A (glycowithanolides) have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidise (GPX) levels in the rat brain frontal cortex and striatum. It was noted that the administration of active glycowithanolides of ashwagandha (10 or 20 mg/kg, i.p for 21 days) increases levels of all the enzymes(12). This implies that ashwagandha does have an antioxidant effect in the rat brain which is be responsible for its pharmacological properties. In another study, an aqueous suspension of ashwagandha roots extract was evaluated for its effect on stress-induced lipid peroxidation (LPO) in mice and rabbits. It was noted that the blood levels of LPO increased by administration of 0.2 mg/kg of lipopolysaccharides (LPS) from Klebsiella pneumoniae and 100 mg/kg of peptidoglycans (PGN) from Staphylococcus aureus. Simultaneous oral administration of Ashwagandha extract (100 mg/kg) prevented the increase in LPO.

Anti-inflammatory effect of Ashwagandha:

The effectiveness of ashwagandha in a variety of rheumatologic conditions due to its anti-inflammatory properties, which has been studied by several authors. Powdered root of WS (1 g/kg suspended in 2% gum acacia, 50 mg/mL) was given orally one hour before the induction of inflammation by injection of Freund's complete adjuvant in rats and continued daily for three days; it was found that WS caused dose-dependent suppression of α2-macroglobulin (an indicator for anti-inflammatory drugs) in the serum of rats inflammed by sub-plantar injection of carrageenan suspension. The doses of WS root powder were 500, 1000, 1500, or 1200 mg/kg given as suspension orally 3-4 hours prior to induction of inflammation. Maximum effect (about 75%) was seen at 1000 mg/kg. Actual measurements of inflammation were not conducted[13].

Antitumor effect of ashwagandha:

To investigate its use in treating various forms of cancer, the antitumor and radiosensitizing effects of WS have been studied. In one study, WS was evaluated for its anti-tumor effect in urethane-induced lung adenomas in adult male albino mice[14]. Simultaneous administration of WS (ethanol extract of whole plant, 200 mg/kg daily orally for seven months) and urethane (125 mg/kg without food biweekly for seven months) reduced tumor incidence significantly (tumor incidence: untreated control, 0/25; urethane treated, 19/19; WS treated, 0/26, and WS plus urethane treated, 6/24, $p<0.05$) The histological appearance of the lungs of animals protected by WS was similar to those observed in the lungs of control animals. No pathological evidence of any neoplastic change was observed in the brain, stomach, kidneys, heart, spleen, of any treated or control animals. In addition to providing protection from carcinogenic effects, WS treatment also reversed the adverse effects of urethane on total leukocyte count, lymphocyte count, body weight, and mortality[15]. The growth inhibitory effect of WS was also observed in Sarcoma 180 (S-180), a transplantable mouse tumor. Ethanol extract of WS root (400 mg/kg and up, daily for 15 days) after intra-dermal inoculation of 5x105 cells of S-180 in BALB/c mice produced complete regression of tumor after the initial growth. A 55-percent complete regression was obtained at 1000 mg/kg; however, it was a lethal dose in some cases. WS was also found to act as a radio- and heat sensitizer in mouse S180 and in Ehrlich ascites

carcinoma. Antitumor and radiosensitizing effects of withaferin (a steroidal lactone of WS) were also seen in mouse Ehrlich ascites carcinoma in vivo. Withaferin A from WS gave a radiosensitizer ratio of 1:5 for in vitro cell killing of V79 Chinese hamster cell at a non-toxic concentration of about 2 mM/L.These studies are suggestive of antitumor activity as well as enhancement of the effects of radiation by WS[(16)].

Estrogenic Activity of Ashwagandha:

The effect of Ashwagandha root extract on Osteoporosis. The ethanolic root extract contains oestrogen-like withanolides for anti-osteoporotic activity. The author observed significant increase in serum (ALP) levels and excretion of urinary Ca and P in withanolide in tested group. Khazal et al. (2013) studied the effect of Ashwagandha root extract on Estrogen Receptor-Positive Mammary Carcinomas. The authors found that in tested group the rate of cell division, in the mammary tumours was significantly reduced[(17)].

Effects of Ashwagandha on the Alzheimer's disease:

The effect of withanoside IV in mice with spinal cord injury (SCI) it was found that in SCI the myelin levels in axons, white matter, gray matter and CNS has decreased. Treatment with withanoside IV (10 µmole/kg body) resulted in increase axonal density with increase myelin levels in peripheral nervous system (PNS); the loss of CNS myelin was not affected. The authors suggested that oral administration of withanolide IV may ameliorate locomotors function by facilitating both axonal regrowth and increase in PNS myelin levels. Konar et al. (2011) reported that administration of scopolamine resulted in down regulation of the expression of BDNF and GFAP in dose and time dependent manner[(18)]. Treatment with alcoholic extract of ashwagandha leaf markedly attenuated these effects. Similarly effects was noted in IMR32 neuronal and C6 glioma cells the authors concluding that scopolamine besides the blocking cholinergic receptors, may induce memory loss by causing oxidative stress; leaf extract of ashwagandha and withanone may serve as potential preventive and therapeutic agents.

Effects of Ashwagandha on the Endocrine System:

The efficacy of ashwagandha in regulating thyroid function and based on the observations. Author suggested that ashwagandha provides protection from free radical damage in the mouse liver. In another study ashwagandha root extract were given to mice (1.4 g/kg, daily for 20 days) and it was noted that the treatment significantly increased the serum levels of 3,3‟,5-triiodothyronine (T3) and tetraiodothyronine (T4), while the hepatic concentrations of glucose 6-phosphatase activity and hepatic iodothyronine 5‟-monodeiodinase activity did not change significantly. Ashwagandha significantly reduced hepatic LPO and increased the activity of SOD and catalase[19]. The results suggest that ashwagandha stimulates thyroidal activity and also promotes hepatic antioxidant activity.

Hemopoetic Effect of Ashwagandha:

Administration of ashwagandha extract was found to significantly reduce leukopenia induced by cyclophosphamide (CTX) treatment in Swiss albino mice (Davis and Kuttan, 1998). Total white blood cell count was in normal range in CTX-plus-Ashwagandha group. In the CTX-plus-Ashwagandha mice, the cellularity of the bone marrow was significantly increased compared to the CTX-alone treated group. Similarly, the number of alpha-esterase positive cells in the bone marrow of the CTX-plus-Ashwagandha in mice increased compared to the CTX alone in mice. The major activity of ashwagandha may be the stimulation of stem cell proliferation. These studies indicated that ashwagandha reduced CTX-induced toxicity and may prove useful in cancer chemotherapy[20].

Immunomodulatory Activities of Ashwagandha:

The use of Ashwagandha as a general tonic to increase energy balance and prevent disease may be partially related to its effect on the immune system. Ghosal et al. (1989) evaluated the immunomodulatory and central nervous system effects (antistress, memory, and learning) of glycowithanolides and a mixture of sitoindosides IX and X isolated from ashwagandha in Swiss mice and Wistar rats. The author observed that both extracts produced significant and activation of peritoneal macrophages, phagocytosis, and increased activity of the lysosomal enzymes; it also produced significant anti-stress activity in mice and memory retention in both young and old rats[21].

Effects of Ashwagandha on Nervous System:

Total alkaloid extract (ashwagandholine, AG) of Ashwagandha roots has been studied for its effects on the central nervous system. AG exhibited a effect on mild depressant (tranquilizer) and effect on the central nervous system in monkeys, cats, dogs, rats and mice. AG had no analgesic activity in rats but increased Metrazol toxicity in rats and mice, amphetamine toxicity in mice, and produced hypothermia in mice(22). It also potentiated barbiturate, ethanol, and urethane induced hypnosis in mice.

1.4 INTRODUCTION OF ETHOSOMES:

Skin is the largest human organ and consists of three functional layers: epidermis, dermis, and subcutaneous(24). It has a wide variety of functions: one major task of the skin is to protect the organism from water loss and mechanical, chemical, microbial and physical environments. The protective properties are provided by the outermost layer of the skin.

Transdermal drug delivery can be used as an alternative delivery of drug into the systemic circulation .Transdermal drug delivery offers many advantages as compared to traditional drug delivery better alternative to achieve constant plasma levels for prolonged periods of time, which additionally could be advantageous because of less frequent dosing regimens.

Advantages clamied are increased patient acceptability,avoidance of first pass metabolisam,predictable and extended duration of activity, minimizing side effects and utility of short half life of drugs ,improving physiological and pharmacological response,avoiding the fluctuation in drug levels .The barrier function govern by stratum corneum is main problem for delivery of drugs across the skin(25).The strateum corneum consists of corneocytes surrounded by lipid layers ,which play an essential role in the barrier properties of the strateum corneum.

In order to increase the number of drugs administered via transdermal route,novel drug delivery systems have to be designed. These systems include use of physical means, such as iontophoresis, sonophoresis, microneedles, etc, and chemical means like penetration enhancers and biochemical means using liposomes,niosomes,transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum.The vesicles have been well known for their importance in cellular communication and particle transportation for many years.Researchers

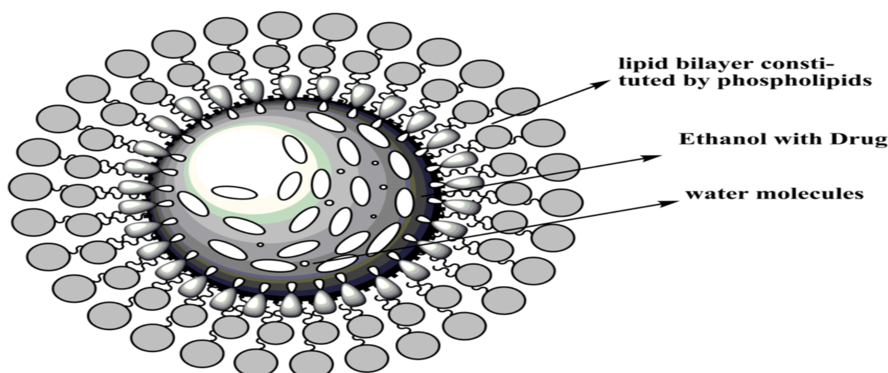
have understood the properties of vesicles structure for use in better drug delivery within their cavities ,which would to tag the vesicle for cell specificity.one of the major advances in vesicle research was the finding a vesicle derivatives known as an ethosomes(26).

ETHOSOMES AS NOVEL CARRIER FOR HERBAL DRUG:

Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers andor the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems be able to release the right amount of drug and keep that concentration constant for longer periods of time(27). One of the major advances in vesicle research was the finding of a vesicle derivative, known as an Ethosomes.

Ethosomes are the slight modification of well established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water(28). Ethosomes are soft vesicles made of phospholipids , ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers (nm) to microns (μ) ethosomes permeate through the skin layers more rapidly possess significantly higher transdermal flux.

Figure 1.1 Structure of ethosome



The ethosomal system is composed of phospholipid, high concentration of alcohol and water. The high concentration of ethanol makes ethosomes unique because ethanol causes disturbance of skin lipid bilayer organization, hence when incorporated into a vesicle membrane, it enhances the vesicles ability to penetrate the stratum corneum.

Composition of Ethosomes:-

Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosph-atidylserine, phosphatitidic acid), high concentration of ethanol and water. The nonaqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer therefore, when integrated into a vesicle membrane, it gives vesicle ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum[29].

Table: 1.4.1 Different additives employed in formulation of ethosomes

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, Distearyl phosphatidyl choline	Vesicles forming component
Alcohol(29)	Ethanol, Isopropyl alcohol	For providing the softness for vesicle membrane as a penetration enhancer
Polyglycol	Propylene glycol, Transcutol RTM	As a skin penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123, Rhodamine red, Fluorescene	For For characterization study

	Isothiocynate, (FITC)6-Carboxyfluorescence	
Vehicle	Carbopol Ð934	As a gel former

1.4.2 Advantages of Ethosomal Drug Delivery

- Delivery of large molecules (peptides, protein molecules) is possible.
- It contains non-toxic raw material in formulation.
- Enhanced permeation of drug through skin for transdermal drug delivery.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance[30].
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.

1.4.3 Disadvantages of Ethosomal Drug Delivery

- Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
- Adhesive may not adhere well to all types of skin.
- May not be economical.
- Poor yield.
- Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.

1.4.4 Mechanism of Drug Penetration

The main advantage of ethosomes over liposomes is the increased permeation of the drug. The mechanism of the drug absorption from ethosomes is not clear[31]. The drug absorption probably occurs in following two phases:

1. Ethanol effect

2. Ethosomes effect

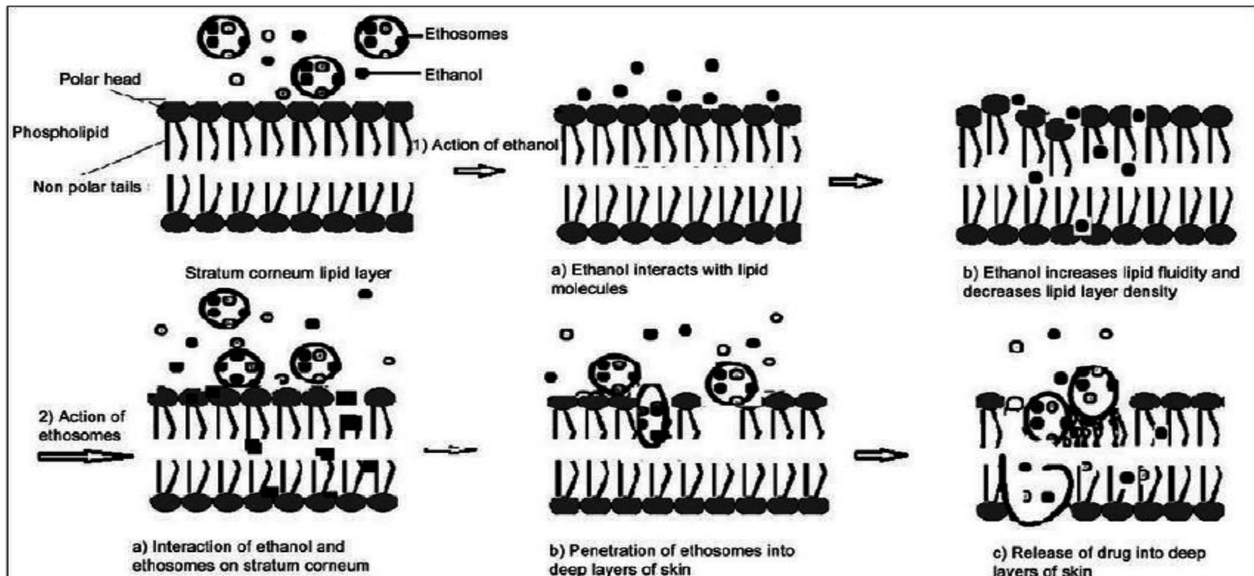
Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

Ethosomes effect

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

Figure 1.3.4 Drug penetration mechanisam of Ethosomes:



1.5 Methods of preparation of Ethosomes

Ethosomes can be prepared by two very simple and convenient methods that is;

1. Cold method
2. Hot method

1. Cold Method

This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer (32). Propylene glycol or other polyol is added during stirring. This mixture is heated to 30^0C in a water bath. The water heated to 30 ^{0}C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

2. Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^0C. Once both mixtures reach 40^0C the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

1.5.1 CHARACTERIZATION OF ETHOSOMES

Visualization of vesicles

Vesicles are visualized by Transmission electron microscopic (TEM) and scanning electron microscopic (SEM).

Vesicle size and zeta potential

Vesicle size is measured by Dynamic light scattering (DLS) and photon correlation spectroscopy(PCS).Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems and it can be measured by zeta meter[33].

Entrapment efficiency:

Entrapment efficiency is determined by Ultracentrifugation technique.

Surface tension activity measurement

It is measured by Ring method in a Du Nouy ring tensiometer.

Transition temperature

It is determined by means of Differential scanning calorimetry.

Penetration and permeation studies

Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM)

Stability of ethosomes

The ability of ethosomal formulations to retain the drug was checked by keeping the preparations at different temperatures, i.e., 25±2°C and 45±2°C for different periods of time.

The stability of ethosomes can also be determined quantitatively by monitorning size and morphology of the vesicles using DLS and TEM.

Degree of deformability and turbidity

The degree of deformability of the ethosomal preparation can be performed by extrusion method and the turbidity of the preparation can be performed by using nephelometer[34]

- **Therapeutics application of Ethosomes**:

Transdermal Delivery of Hormones

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several doses dependent side effects. The risk of failure of treatment is known to increase with each pill missed.

In the treatment herpetic infection

5% acyclovir ethosomal preparation compared to the 5% acyclovir cream showed siginificant improvements in treatment of herpetic infections.

Delivery of Anti-Arthritis Drug

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery andovercomes the problem associated with conventional oral therapy [35].

Transcellular Delivery-

Ethosomesas compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

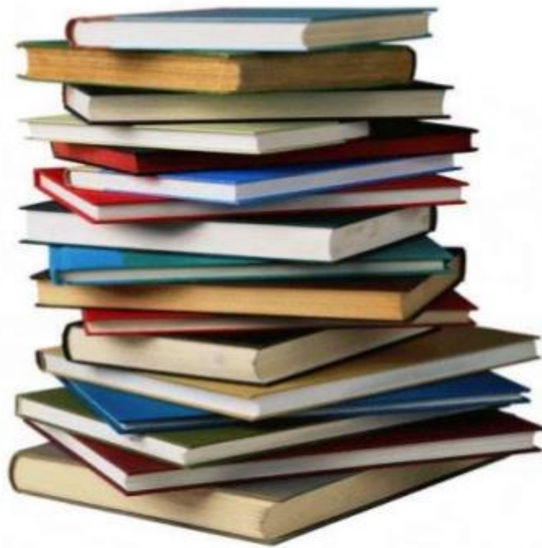
Ethosomes are used in pilosabeceous targeting Ethosomes the high ethanol containg vesicles are able to penetrate deeper layers of the skin and hence appear to be vesicles of choices for transdermal drug delivery of hydrophilic and impermeable drugs through the skin.

Table:-1.5.2 Marketed Products Based On Ethosomal Drug Delivery System

Name of product	Uses	Manufacturer
Cellutight EF	Topical cellulite cream, contains a powerful combination of ingredients to increase metabolism and break down fat	Hampden Health, USA
Decorin cream	Anti-aging cream, treating, repairing, and delaying the visible aging signs of the skin including wrinkle lines,	Genome Cosmetics, Pennsylvania, US

	sagging, age spots, loss of elasticity, and hyper pigmentation	
Nanominox	First minoxidil containing product, which uses ethosomes. Contains 4% Minoxidil, well-known hair growth promoter that must be metabolized by sulfation to the active compound.	Sinere, Germany
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
Skin genuity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK
Supravir cream	For the treatment of herpes virus	Trima,Israel

CHAPTER - 2
LITERATURE
REVIEW



2.Literature review

2.1 General work

1.TAWONA.N et al., 2017 has worked on topical delivery of withania somnifera crude extracts in niosomes and solid lipid nanoparticles. Solvent injection method was utilized for the formulation of niosomes and SLN. Ethanol, methanol, high performance liquid chromatography, acetonitrile and chloroform. SLNs and niosomes were able to encapsulate crude extracts of W. somnifera and release the marker compounds, withaferin A and withanolide A, for delivery to certain layers in the skin. Highest percentage encapsulation (95.3%) that was obtained for withanolide A from the water extract SLNs. Withania somnifera is a medicinal plant native to India and is known to have anticancer properties. It has been investigated for its anti-melanoma properties, and since melanoma presents on the skin, it is prudent to probe the use of W. somnifera in topical formulations. To enhance topical drug delivery and to allow for controlled release, the use of niosomes and solid lipid nanoparticles (SLNs) as delivery vesicles were explored. The objective of this study is to determine the stability and topical delivery of W. somnifera crude extracts encapsulated in niosomes and SLNs. Materials and methods: Water, ethanol, and 50% ethanol crude extracts of W. somnifera were prepared using 24 h soxhlet extraction which were each encapsulated in niosomes and SLNs. Franz cell diffusion studies were conducted with the encapsulated extracts to determine the release and skin penetration of the phytomolecules, withaferin A, and withanolide A. The niosome and SLN formulations had average sizes ranging from 165.9 ± 9.4 to 304.6 ± 52.4 nm with the 50% ethanol extract formulations having the largest size. A small particle size seemed to have correlated with a low encapsulation efficiency (EE) of withaferin A, but a high EE of withanolide A. There was a significant difference ($P < 0.05$) between the amount of withaferin A and withanolide A that were released from each of the formulations, but only the SLN formulations managed to deliver withaferin A to the stratum corneum-epidermis and epidermis-dermis layers of the skin. SLNs and niosomes were able to encapsulate crude extracts of W. somnifera and release the marker compounds, withaferin A, and withanolide A, for delivery to certain layers in the skin(36).

2. BHASKAR GANGULY et al., 2017 has worked on influence of phytochemical composition on in vitro antioxidant and reducing activities of indian ginseng [withania somnifera (l.) dunal]

root extracts. Roots of *Withania somnifera (WS)* are a medicinal ingredient in Ayurvedic and many other indigenous systems of medicine. The present study investigates the effect of the phytochemical composition of the extracts on their antioxidant and reducing activities. WS roots were extracted with water, acetone, aqueous methanol (1:1), and methanol: chloroform :water (1:1:1) to obtain aqueous, acetone, hydro-methanolic, and methanol–chloroform–water extracts. Thereafter, phytochemical constitution and antioxidant and reducing activities of the extracts were compared using different qualitative and quantitative tests. Maximum extraction recovery was obtained with 50% aqueous methanol whereas extraction with acetone yielded the poorest recovery. Methanol–chloroform–water extract had the highest content of phytochemical constituents, except tannins, and also exhibited the highest antioxidant and reducing activities(37). Alkaloids and flavonoids were the most important contributors in the antioxidant and reducing activities of the extracts.

3. AINE BRIGETTE HENLEY et al.,2017 has worked on Withania somnifera Root Extract Enhances Chemotherapy through ‘Priming’. Withania somnifera extracts are known for their anti-cancerous, anti-inflammatory and antioxidative properties. One of their mechanisms of actions is to modulate mitochondrial function through increasing oxidative stress. Recently ‘priming’ has been suggested as a potential mechanism for enhancing cancer cell death. In this study we demonstrate that ‘priming’, in HT-29 colon cells, with *W. somnifera* root extract increased the potency of the chemotherapeutic agent cisplatin. We have also showed the *W. somnifera* root extract enhanced mitochondrial dysfunction and that the underlying mechanism of ‘priming’ was selectively through increased ROS. Moreover, we showed that this effect was not seen in non-cancerous cells. Method: *Withania somnifera* root powder was shaken with 2 mL of dilute ammonia R4. Methanol (20 mL) was added and the mixture was sonicated for 20 minutes. It was then heated on the water bath for 3 minutes and filtered(38). The filtrate was evaporated to dryness at 60°C. A stock solution of 0.08335 g of dry extract /mL DMSO was prepared for biological studies.

4. RAMIN NASIMI DOOST AZGOMI et al.,2017 has worked on Effects of Withania somnifera on Reproductive System. Introduction. Withania somnifera (WS) also known as ashwagandha is a well-known medicinal plant used in traditional medicine in many countries for infertility treatment. The present study was aimed at systemically reviewing therapeutic effects of

WS on the reproductive system. Methods. This systematic review study was designed in 2016. Required data were obtained from PubMed, Scopus, Google Scholar, Cochrane Library, Science Direct, Web of Knowledge, Web of Science, and manual search of articles, grey literature, reference checking, and expert contact. Results. WS was found to improve reproductive system function by many ways. WS extract decreased infertility among male subjects, due to the enhancement in semen quality which is proposed due to the enhanced enzymatic activity in seminal plasma and decreasing oxidative stress. Also, WS extract improved luteinizing hormone and follicular stimulating hormone balance leading to folliculogenesis and increased gonadal weight, although some animal studies had concluded that WS had reversible spermicidal and infertilizing effects in male subjects. Conclusion. WS was found to enhance spermatogenesis and sperm related indices in male and sexual behaviors in female. But, according to some available evidences for spermicidal features, further studies should focus on the extract preparation method and also dosage used in their study protocols(39).

5. HODA M FATHY et al.,2017 has Validated on thin-layer chromatographic method for the identification and monitoring of the effect of the extraction method on the yield and phytochemical constituents of Egyptian Withania somnifera leaves. A sensitive, reliable, simple and rapid thin-layer chromatographic method has been developed for routine analysis of withanolide S content for the purpose of quality control assessment of chemotype III of W. somnifera. The new method was used first to compare the accumulation of withanolide S in different parts of the plant, which was found to be the highest in the leaves extract (0.21% w/w). Secondly to investigate different extraction parameters that improve the extraction efficiency of withanolides from the leaves using conventional and ultrasound-assisted extraction methods(40). The extraction efficiency was expressed via total withanolide content (Twc) and withanolide S content.

6. VIBHA PANDEY et al.,2017 has worked on Withania somnifera: Advances and Implementation of Molecular and Tissue Culture Techniques to Enhance Its Application. Withania somnifera, commonly known as Ashwagandha an important medicinal plant largely used in Ayurvedic and indigenous medicine for over 3,000 years. Being a medicinal plant, dried powder, crude extract as well as purified metabolies of the plant has shown promising therapeutic properties. Withanolides are the principal metabolites, responsible for the medicinal properties of the plant(41). Availability and amount of particular withanolides differ with tissue type and

chemotype and its importance leads to identification characterization of several genes/ enzymes related to withanolide biosynthetic pathway. The modulation in withanolides can be achieved by controlling the environmental conditions like, different tissue culture techniques, altered media compositions, use of elicitors, etc. Among all the *in vitro* techniques, hairy root culture proved its importance at industrial scale, which also gets benefits due to more accumulation (amount and number) of withanolides in roots tissues of *W. somnifera.* Use of media compostion and elicitors further enhances the amount of withanolides in hairy roots. Another important modern day technique used for accumulation of desired secondary metabolites is modulating the gene expression by altering environmental conditions (use of different media composition, elicitors, etc.) or through genetic enginnering. These standardized transformation procedues have been used to overexpress/silence desired gene in *W. somnifera* to understand the outcome and succeed with enhanced metabolic production for the ultimate benefit of human race.

7 . DHANANJAY DWIVED et al.,2015 has worked on study of phytochemical active compounds in extract of withania somnifera. Leaves of withania somnifera was allowed to potassium dihydrogen phosphate, phosphoric acid reflux in methanol for 3hrs.chemicals phosphoric acid and acetonitrile. The plant under testing possessed greater potential anticancer as well as anti- diabetic property. The bioactive content was isolated and extracted and was analyzed by HPLC and the observations and pharmacological property were discussed above. The studies for Withania somnifera would be good natural source of potent and chemotherapeutic agent(42). Ashwagandha is a plant used in medicine form the time of Ayurveda, since long time in India. Ashwagandha has been used as an aphrodisiac, anti-inflammatory agent, astringent, asthma, ulcers, and insomnia. Studies on animal shows the plant is used for treatment of neurological disorders, inflammation and Parkinson’s disease.

8. SHRUTI SINGH et al., 2015 has worked on Withania somnifera root extract has found that Potent Cytotoxic Effect against Human Malignant Melanoma Cells. In Ayurveda, Withania somnifera is commonly known as Ashwagandha, its roots are specifically used in medicinal and clinical applications. It possesses numerous therapeutic actions which include anti-inflammatory, sedative, hypnotic and narcotic. Extracts from this plant have been reported for its anticancer properties. In this study we evaluated for the first time, the cytotoxic effect of *Withania* root extract

on human malignant melanoma A375 cells[43]. The crude extract of *Withania* was tested for cytotoxicity against A375 cells by MTT assay. Cell morphology of treated A375 cells was visualized through phase contrast as well as fluorescence microscopy. Agarose gel electrophoresis was used to check DNA fragmentation of the crude extract treated cells. Crude extract of *Withania* root has the potency to reduce viable cell count in dose as well as time dependent manner. Morphological change of the A375 cells was also observed in treated groups in comparison to untreated or vehicle treated control. Apoptotic body and nuclear blabbing were observed in DAPI stained treated cells under fluorescence microscope. A ladder of fragmented DNA was noticed in treated cells. Thus it might be said that the crude water extract of Withania somnifera has potent cytotoxic effect on human malignant melanoma A375 cells. Method Five gram of dried powder of root of *Withania somnifera* was taken and soaked overnight in 50ml of deionized water. After that it was stirred with the help of magnetic stirrer continuously for about 72 hr followed by centrifugation. The supernatant was then used for further experimentation.

9. ANISHA BANO et al.,2015 has prepared A Systematic and Comprehensive Review on Withania somnifera (L.) Dunal- An Indian Ginseng. Present review article reveals the importance of species Withania somnifera (L.) Dunal, distributed in India and other parts of the world, this extensive research information on this species is highly significant for future researchers worldwide. In this article cytomorphological, phytochemical and biological activities inputs have been extensively recorded and discussed. As a part of our investigation on cytomorphological and phytochemical aspects for important medicinal plants from India, the aim of this pioneer attempt is to provide precise, truthful and detailed information of W. somnifera (L.). As per our knowledge, there is not even a single, combined, constructive review report available about this species, evaluated by using cytomorphological, phytochemical and biological activities based aspects[44].

10. VIKAS KUMAR et al., 2015 has investigated on Chemistry and pharmacology of Withania somnifera. Withania somnifera (Ashwagandha) is an important Rasayana herb and widely considered as Indian ginseng in Ayurveda. In traditional system of Indian medicine, it is used as tonic to rejuvenate the body and increase longevity. In Ayurvedic preparations, various parts of the plant have been used to treat variety of ailments that affect the human health. However, dried roots of the plant are widely used for the treatment of nervous and sexual disorders. The major active chemical constit uents of this plant are withanolides, which is responsible for its wide range

of biological activities. Since the beginning of the 20 th century, a significant amount of research has been done and efforts are ongoing to further explore other bioactive constituents, and many pharmacological studies have been carried out to describe their disease preventing mechanisms. In this chapter, we have reviewed the chemistry and pharmacological basis of W. somnifera in various human ailments[45].

11. LAXMAN P. SAWANT et al., 2014 has worked on Development and Validation of High Performance Liquid Chromatography Method for Simultaneous Estimation of Flavonoid Glycosides in Withania somnifera Aerial Parts. Withania somnifera (L.) Dunal (Solanaceae) commonly known as ashwagandha, is an important plant in Ayurveda and is believed to increase longevity and vitality(46). The root is considered to be the medicinally important part of the plant as per classical texts and accordingly is the subject of most Pharmacopeial monographs. The aerial parts, being less expensive, are sometimes mixed with roots to prepare "standardized" extracts of W. somnifera, and in cases with false declaration of plant part used as roots on the certificate of analysis. The present study described a new, simple, accurate, and precise HPLC method for the simultaneous determination of flavonoid glycosides as unique constituents of the aerial parts, being absent in roots of the plant. The RSD for intra- and interday analyses was less than 2.5% and the recovery was 90–108%. The method was used to analyze samples of roots and aerial parts of the plant collected from India and Egypt. The samples of commercially available extracts of W. somnifera were also analyzed and many samples were found to contain flavonoid glycosides indicating a possible undeclared use of aerial parts in the extracts derived from roots in commercial practice.

12. PRASUNA SUNDARI PINGALI et al.,2014 has worked on Formulation and Evaluation of Capsules of Ashwagandha Phytosomes. Reflux method. Materials: soya lecithin, Potassium ferricyanide, ferric chloride ,ethanol were used. It was concluded that Ashwagandha phytosomes serve as useful novel drug delivery system and provide more bioavailability than conventional formulations. Objective of the present study was to formulate and evaluate capsules of Ashwagandha phytosomes. There are many herbal extracts having excellent in-vitro activity but less in-vivo activity because of their macromolecular size and poor lipid solubility, which result in poor absorption and bioavailability problems[47]. Many of these problems can be overcome by

formulating novel drug delivery systems. Phytosomes provide better absorption and bioavailability than the conventional herbal extracts. This project aims in improving the drug release characteristics of Ashwagandha by formulating Ashwagandha phytosome capsules. Ashwagandha Phytosomes were produced by a process in which standardized plant extract was bound to phospholipids, producing a lipid compatible molecular complex. Ashwagandha phytosome complexes were characterized by particle size, zeta potential, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and in vitro drug release. The results showed that the average particle size and zeta potential of optimized Ashwagandha phytosomes formulation were 98.4nm and −28.7 mV. In vitro drug release studies revealed that the cumulative % drug release of capsules of Ashwagandha phytosomes was found to be 76.8%. Antioxidant activity of Ashwagandha phytosomes was evaluated by reducing power method. The results showed that the Ashwagandha phytosome complex exhibited more antioxidant activity compared to the Ashwagandha extract. Hence it was concluded that Ashwagandha phytosomes serve as useful novel drug delivery system and provide more bioavailability than conventional formulations.

13. MONIKA JOON et al.,2013 has worked on Formulation and Evaluation of Standardised Withania somnifera Leaf Extract Loaded Transdermal Gel. Proniosomes gel was prepared by coacervation phase separation method. Materials: acetonitrile, potassium dihydrogen phosphate, cholesterol, ethanol and span 60.the method used for the preparation of proniosomal gel result in the higher drug entrapment value of 87.2%. Scanning electron microscopy showed that the surface of the particles are smooth.the formulations showed prolonged invitro drug release 60.8% over a period of 24h and significant anti-inflammatory response. It is concluded that proniosomes are very stable and promising delivery system for Withania somnifera leaf extract.

14. SHEBA RANI NAKKA DAVID et al**.,2013** has worked on Formulation and in vitro evaluation of ethosomes as vesicular carrier for enhanced topical delivery of isotretinoin Isotretinion ethosomal formulation were prepared by "Hot method"Materials : soyalecithin, ethanol, propylene glycol. It is concluded that the ethosomal vesicles and enhancers increased the skin permeation and depot formation of drug in the skin. The purpose of the present research was to evaluate the ability of ethosomes for topical delivery of isotretinoin. The ethosomal vesicles were prepared with various concentrations of lecithin and ethanol by using hot method. The ethosomal based isotretinoin gel (GEL-ES) was compared to that of marketed formulations

isotretinoin (GEL-MF) by using hydrophobic hydroxyl propyl methyl cellulose as gel base(48). The physicochemical and stability of ethosomal based isotretinoin and a marketed gel (control) were evaluated for organoleptic properties, drug entrapment, drug content uniformity and in vitro drug release and skin permeation studies. F2 ethosomal vesicles containing 2%w/w lecithin and 30%w/w ethanol was found to have shown the best entrapment percentage (99.21%) and also showed suitable physicochemical characteristics for topical administration. Physical stability studies were also conducted for 45 days at 4 C and 25 C. GEL-ES and GEL-MF were applied to rat skin and penetration was assessed by Franz diffusion cells. In vitro release studies showed that less than 10% of isotretinoin reached the receptor compartment compared to GEL-MF till 8 h. On comparing F2 and F4 gel formulations, F2 gel has shown better controlled release by in vitro drug release and in vitro skin permeation profile than F4 gel. However, the in vitro skin permeation was increased with the addition of enhancers. From the experimental data, it may be concluded that the ethosomal vesicles and enhancers increased the skin permeation and depot formation of drug in the skin.The entrapment efficiency - 78.53%.

15. YULIN REN et al.,2012 has workedon Isolation and characterization of a bactericidal withanolide from *Physalis virginiana. Physalis virginiana* (Virginia Groundcherry) is a member of the family Solenaceae. Several species of the ***physalis*** genus have been used traditionally by American Indians as medicinal treatments. This study investigated the antibacterial activity of chemicals extracted from ***P. virginiana*** through antibacterial disc and cytotoxicity assays. Isolation and purification of an antimicrobial compound was achieved through flash chromatography and preparative HPLC. Finally, identification of chemical structure was determined from ^{1}H and ^{13}C NMR and MS. Disc assays showed that crude ethanol extracts were effective antibacterial agents against one gram-negative and seven gram-positive bacterial strains. Cytotoxicity assays indicated that it is less toxic than gentamicin controls. Isolation of the active component showed it to be a relatively polar compound. ^{1}H and ^{13}C NMR chemical shifts together with HRMS indicated a similar structure to withanolides previously identified from Physalis angulata. HRMS analysis showed a molecular mass of 472.2857 which corresponds to a molecular formula $C_{28}H_{40}O_{6}$. An antibacterial withanolide was isolated from ***P. virginiana*** using flash chromatography and HPLC separations. The chemical structure was determined by NMR and MS to be the withanolide physagulin V.

16. QAMAR UDDIN et al., 2012 has worked on Phytochemical and Pharmacological Profile of Withania somnifera Dunal. Withania somnifera (L) Dunal is a well known Indian medicinal plant widely used in the treatment of many clinical conditions in India. It is an important drug commonly known as Asgand which has been used either single or in combination with other drugs in Unani as well as Ayurvedic system of medicine for centuries. It has been described by Dioscorides (78 AD) in his book "Kitab-ul-Hashaish". Asgand consists of the roots of Withania somnifera which has various therapeutic actions such as anti-inflammatory (Muhallil-e-Warm), sedative (Musakkin), alterative (Muaddil) and aphrodisiac (Muqawwi-e-Bah)(49). Keeping in view the medicinal properties of Withania somnifera Dunal (Asgand), an attempt has been made in this review paper to explore various dimensions of the drug including phytochemical and pharmacological studies carried out on this drug.

17. SANTOSH T DEVKER et al.,2012 has isolated An antibacterial withanolide was isolated from ***P. virginiana*** using flash chromatography and HPLC separations. The chemical structure was determined by NMR and MS to be the withanolide physagulin V. The high-performance thin-layer chromatography (HPTLC) method has been developed for the simultaneous quantification of withaferine A, 1,2 deoxy-withastramonolide, withanolide A, and withanolide B for the validation of Withania somnifera (Ashwagandha roots) as raw material and Ashwagandha-containing finished Ayurvedic products. HPTLC of W. somnifera methanolic extracts was performed on Si 60F(254) (10 x 10 cm) HPTLC plates with dichloromethane-toluene-methanol-acetone-diethyl ether (7.5:7.5:3:1:1 v/v) as a mobile phase. Upon separation, quantitative evaluation of these withanolides was performed in the absorption reflection mode at 235 nm. The method was validated for precision, reproducibility, and accuracy. On the basis of R-F values of 0.58, 0.61, 0.68, and 0.79 for withaferine A, 1,2 deoxy-withastramonolide, withanolide A, and withanolide B, respectively, were identified. On the basis of linear calibration curves for all withanolides in the range of 0.2-1.2 mu g, an average recovery of withaferine A, 1,2 deoxy-withastramonolide, withanolide A, and withanolide B was 98%, 99.5%, 98%, and 99%, respectively. The method is very simple, precise, specific, sensitive, accurate, and economical for rapidly validating the Ayurvedic products containing W. somnifera (Ashwagandha).

18. MR AGRAWAL et al.,2011 has worked on Simultaneous Determination of Withanolide A and Bacoside A in Spansules by High-Performance Thin-Layer Chromatography**.** The objective

of this work was to develop and validate a simple, rapid, precise, and accurate high performance thin layer chromatography method for simultaneous determination of withanolide A and bacoside A in combined dosage form. The stationary phase used was silica gel $G60F_{254}$. The mobile phase used was mixture of ethyl acetate: methanol: toluene: water (4:1:1:0.5 v/v/v/v). The detection of spots was carried out at 320 nm using absorbance reflectance mode. The method was validated in terms of linearity, accuracy, precision and specificity(50). The calibration curve was found to be linear between 200 to 800 ng/spot for withanolide A and 50 to 350 ng/spot for bacoside A. The limit of detection and limit of quantification for the withanolide A were found to be 3.05 and 10.06 ng/spot, respectively and for bacoside A 8.3 and 27.39 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

19. NARENDRA SINGH et al.,2011 has worked on Ashwagandha: A Rasayana (Rejuvenator) of Ayurveda. *Withania somnifera* (Ashawagandha) is very revered herb of the Indian Ayurvedic system of medicine as a Rasayana (tonic). It is used for various kinds of disease processes and specially as a nervine tonic. Considering these facts many scientific studies were carried out and its adaptogenic / anti-stress activities were studied in detail. In experimental models it increases the stamina of rats during swimming endurance test and prevented adrenal gland changes of ascorbic acid and cortisol content produce by swimming stress. Pretreatment with Withania somnifera *(WS)* showed significance protection against stress induced gastric ulcers. *WS* have anti-tumor effect on Chinese Hamster Ovary (CHO) cell carcinoma. It was also found effective against urethane induced lung-adenoma in mice. In some cases of uterine fibroids, dermatosarcoma, long term treatment with *WS* controlled the condition. It has a Cognition Promoting Effect and was useful in children with memory deficit and in old age people loss of memory. It was also found useful in neurodegenerative diseases such as Parkinson's, Huntington's and Alzeimer's diseases. It has GABA mimetic effect and was shown to promote formation of dendrites. It has anxiolytic effect and improves energy levels and mitochondrial health. It is an anti-inflammatory and anti-arthritic agent and was found useful in clinical cases of Rheumatoid and Osteoarthritis. Large scale studies are needed to prove its clinical efficacy in stress related disorders, neuronal disorders and cancers.

20. PRAKASH KHARE et al.,2011 has worked on Isolation, identification and antimicrobial activity of withanolide WS-1 from roots of Withania somnifera. Column chromatographic purification of the organic extract obtained from the roots of Withania somnifera yielded a biologically active withanolide compound WS-1 whose structure was established on the basis of spectroscopic evidences. Potent antibacterial activity was observed in this compound. The dried plant materials were powdered (1.5 kg) and was first extracted with methanol (7.5 l) in a Sohxlet extractor(51). The solvent was evaporated under reduced pressure in a rotatory evaporator to get methanol extract. Fractions eluted with 60% ethyl acetate in hexane in column chromatography were found identical in TLC with single spot and hence these fractions were mixed and concentrated under reduced pressure. The concentrated white mass was washed with hexane and ether successively. This was recrystallized from alcohol and analyzed as WS**-1.**

21. P B SHINDHE et al.,2011 has investigated on Simultaneous Determination of Withanolide A and Bacoside A in Spansules by High-Performance Thin-Layer Chromatography. The objective of this work was to develop and validate a simple, rapid, precise, and accurate high performance thin layer chromatography method for simultaneous determination of withanolide A and bacoside A in combined dosage form. The stationary phase used was silica gel $G60F_{254}$. The mobile phase used was mixture of ethyl acetate: methanol: toluene: water (4:1:1:0.5 v/v/v/v). The detection of spots was carried out at 320 nm using absorbance reflectance mode. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 800 ng/spot for withanolide A and 50 to 350 ng/spot for bacoside A. The limit of detection and limit of quantification for the withanolide A were found to be 3.05 and 10.06 ng/spot, respectively and for bacoside A 8.3 and 27.39 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

22. ELISABETH MOYANO et al 2009 has investigated on Steroidal Lactones from Withania somnifera, an Ancient Plant for Novel Medicine. Withania somnifera, commonly known as Ashwagandha, is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years. In view of its varied therapeutic potential, it has also been the subject of considerable modern scientific attention. The major chemical constituents of

the Withania genus, the withanolides, are a group of naturally occurring C28-steroidal lactone triterpenoids built on an intact or rearranged ergostane framework, in which C-22 and C-26 are appropriately oxidized to form a six-membered lactone ring. In recent years, numerous pharmacological investigations have been carried out into the components of W. somnifera extracts. We present here an overview of the chemical structures of triterpenoid components and their biological activity, focusing on two novel activities, tumor inhibition and antiangiogenic properties of withaferin A and the effects of withanolide A on Alzheimer's disease. The most recent attempts in biotechnological production of withanolides are also discussed.(52)

23. SRIVASTAVA et al.,2008 has investigated Simultaneous quantification of withanolides in Withania somnifera by a validated high-performance thin-layer chromatographic method**.** This paper describes a sensitive, selective, specific, robust, and validated densitometric high-performance thin-layer chromatographic (HPTLC) method for the simultaneous determination of 3 key withanolides, namely, withaferin-A, 12-deoxywithastramonolide, and withanolide-A, in Ashwagandha (Withania somnifera) plant samples. The separation was performed on aluminum-backed silica gel 60F254 HPTLC plates using dichloromethane-methanol-acetone-diethyl ether (15 + 1 + 1 + 1, v/v/v/v) as the mobile phase. The withanolides were quantified by densitometry in the reflection/absorption mode at 230 nm. Precise and accurate quantification could be performed in the linear working concentration range of 66-330 ng/band with good correlation (r2 = 0.997, 0.999, and 0.996, respectively). The method was validated for recovery, precision, accuracy, robustness, limit of detection, limit of quantitation, and specificity according to International Conference on Harmonization guidelines. Specificity of quantification was confirmed using retention factor (Rf) values, UV-Vis spectral correlation, and electrospray ionization mass spectra of marker compounds in sample tracks.

24.LAKSHMI-CHANDRA MISHRA et al.,2000 has worked on Scientific Basis for the Therapeutic Use of Withania somnifera.The objective is to review the literature regarding Withania somnifera (ashwagandha, WS) a commonly used herb in Ayurvedic medicine. Studies indicate ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems. The mechanisms of action for these properties are not fully understood. Toxicity studies reveal that ashwagandha appears to be a safe compound(53). Preliminary studies have found various constituents of

ashwagandha exhibit a variety of therapeutic effects with little or no associated toxicity. These results are very encouraging and indicate this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects. Clinical trials using ashwagandha for a variety of conditions should also be conducted.

2.2 CONCLUSION DRAWN FROM LITERATURE REVIEW:

- From the literature review it has been observed that:
- The withania somnifera niosome and SLN formulations had a small particle size seemed to have correlated with a low encapsulation efficiency of withaferin A, but a high encapsulation efficiency of withanolide A, but only the SLN formulations managed to deliver withaferin A to the stratum corneum-epidermis and epidermis-dermis layers of the skin.
- Ashwagandha phytosome complex exhibited more antioxidant activity compared to the Ashwagandha extract. In vitro drug release studies revealed that the cumulative % drug release of capsules of Ashwagandha phytosomes was found to be 76.8%.
- Ashwagandha proniosomes showed prolonged in vitro drug release of 60.8% over a period of 24 h and significant anti-inflammatory response.
- It was observed that there was no ethosomal formulation for withania somnifera.
- It was observed that there was poor permeation of withanolide into deeper layers of skin. So, by developing ethosomal formulation the permeation of withanolide in the deeper layers of the skin enhances.

2.3: NEED OF THE STUDY:

- Though withania somnifera posses wide properties like antitumour, anti-inflammatory, antiarthritic and antibacterial etc basic problem is poor bioavailability.

- By converting withania somnifera into vesicular drug delivery system ethosomes which results in reduction of particle size will enhanced penetration through skin.

- By formulating it as transdermal, both systemic and local effect can be obtained.

2.4: AIM AND OBJECTIVE

Aim: The aim of the study is to prepare withania somnifera loaded ethosomes by cold and hot method.

Objectives:

- The objectives of the present research work is to develop ethosomal formulation of withania somnifera.
- To prepare ethosomal formulation of Withania somnifera by cold method and hot method.
- To perform various evaluation studies which includes particle size ,zeta potential , entrapment efficiency, drug content and in vitro diffusion studies for the prepared ethosomal formulations.

CHAPTER -3

MATERIALS AND METHODOLOGY

3. METHODOLOGY

3.1 MATERIALS USED: All the materials and Equipments used in the formulation, evaluation and other experiments are given below.

TABLE NO. 3.1: LIST OF ALL CHEMICALS USED

Name of the chemicals	Sources
Roots of Withania somnifera	Bought from the Ayurvedic medical store
Ethanol	Sigma-Aldrich Corporation.
Triethanolamine	SD Fine Chem. Limited, Mumbai
Propylene glycol	SD Fine Chem. Limited, Mumbai
Methyl paraben	SD Fine Chem. Limited, Mumbai
Propyl paraben	SD Fine Chem. Limited, Mumbai
Methanol	SD Fine Chem. Limited, Mumbai

LIST OF EQUIPMENTS:

Table 3.2: List of all equipment's used

Name of the equipment	Sources
Mechanical Stirrer	REMI Electrotrchnik, Vasai
Vacuum Rotary Evaporator	SECOR, Mumbai.
High Speed Research Centrifuge	Eltek, Mumbai
Analytical balance	Contech Instruent Ltd., Mumbai.
Magnetic Stirrer 2MLH	REMI Electrotrchnik, Vasai
Laboratory Centrifuge	Labtech Equipment, Mumbai.
pH meter	ELICO® LI 120, Vasai.
Water bath	SECOR, Mumbai.
UV Visible Spectrophotometer	ELICO, Mumbai.
Zeta Nano Sizer	Malvern Zetasizer, Malvern UK.
Particle Size Analyser	Malvern Zetasizer, Malvern UK.

3.3 PLANT PROFILE:

Withania somnifera is an important herb from the Ayurvedic medical system used for the treatment of debility, emaciation, impotence and premature ageing. It is also called as 'Indian ginseng'. Its Indian name, ashwagandha, is said to refer to the 'smell and strength of a horse' and possibly alludes to its reputed aphrodisiac properties, although it could also relate to the smell of the root[(54)]. Pharmacological research on Withania has stressed its antitumour and adaptogenic actions, reinforcing its comparison with Panax ginseng. However, Withania occupies an important place in the herbal materia medica because, while it is not as potent as Panax, it lacks the potential stimulating effects of the latter. In fact, it has a mild sedative action as indicated by its specific name 'somnifera'. It is therefore ideally suited to the treatment of overactive but debilitated

patients, in whom Panax might tend to aggravate the overstimulation. Many parts of the plant have been used in traditional medicine, including the leaves, bark and root. Except where specified in this monograph, 'Withania' refers to use of the root. Botanical description: WS is a small, woody shrub in the Solanaceae family that grows about two feet in height. It can be found growing in Africa, the Mediterranean, and India. An erect, evergreen, tomentose shrub, 30-150 cm high, found throughout the drier parts of India in waste places and on bunds. Roots are stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish or lubrid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The roots are the main portions of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring.

3.4 Discription of Withania Somnifera:

It is an evergreen, erect, branching small shrub. It grows as a short shrub (35–75 cm)

Leaves: Simple, glabrous and ovate, up to 10 cm in length.

Stem: Stellate central stem with radial secondary branching and densely covered with wooly hair.

Flowers: Small and green or yellow, about 1 cm in length**.**

Fruits: Globose berries, orange-red when ripe, 6 mm in diameter, enclosed in membranous persistent calyx with milk-coagulating properties.

Seeds: yellow, reniform (2.5 mm diameter).

Roots: Tuberous and long, brown in color with medicinal purposes.In southern Africa the flowering time is mostly from October to June, while the fruiting time is mostly from October to July.

TABLE NO. 3.5:Vernacular names of withania somnifera

English	Winter cherry
Sanskrit	Ashwagandha, Turangi-gandha
Hindi	Punir, asgandh
Bengali	Ashvagandha
Gujrati	Ghodakun, Ghoda, Asoda, Asan
Telgu	Pulivendram, Panneru-gadda, panneru
Tamil	Amukkura, amkulang, amukkuram-kilangu, aswagandhi,
Karnataka	Viremaddlinagadde, Pannaeru, aswagandhi, Kiremallinagida
Goa	Fatarfoda
Punjabi	A Asgand, isgand
Bombay	Asgund, asvagandha
Rajasthani	Chirpotan

TABLE NO. 3.6: PHARMACOGNOSY OF WITHANIA SOMNIFERA

Taxonomy of withania somnifera	
Kingdom	Plantae, Plants
Subkingdom	Tracheobionta, Vascular plants
Super division	Spermatophyta, Seeds plants

Division	Angiosperma
Class	Dicotyledons
Order	Tubiflorae
Family	Solanaceae
Genus	Withania
Species	somnifera Duna

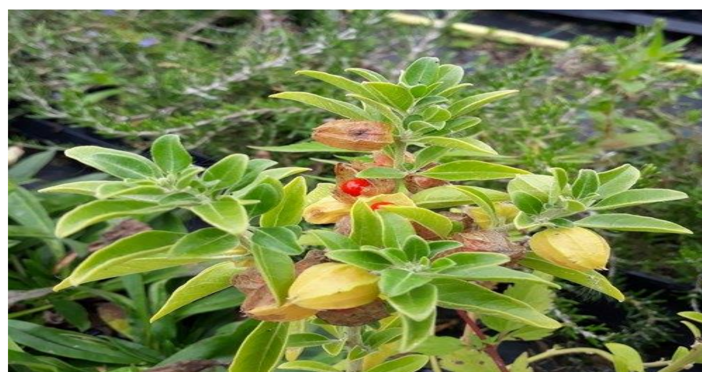


Fig 3.6: Withania somnifera

Fig no 3.6.1: Roots of Withania somnifera

Chemical constituents of withania somnifera:

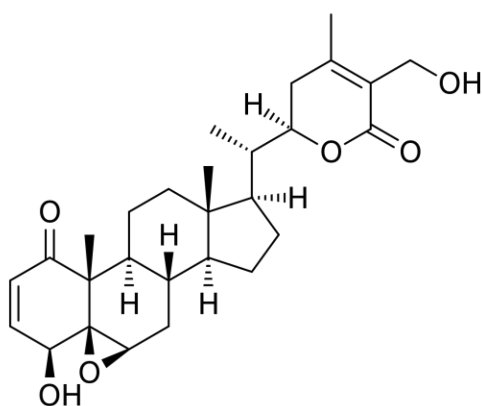
The biologically active chemical constituents of Withania somnifera (WS) include alkaloids (anaferine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins.Sitoindosides in Ashwagandha are anti-stress agents.

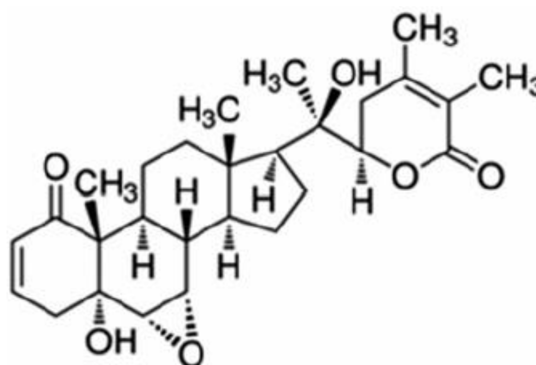
Active principles of Ashwagandha, for instance the sitoindosides and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress .Many of its constituents support immunomodulatory actions(55) .

The aerial parts of Withania somnifera yielded 5-dehydroxy withanolide-R and withasomniferin-A .The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds, including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27.

Apart from these contents plant also contain chemical constituents like withaniol, acylsteryl glucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron.

The biologically active chemical constituents of Withania somnifera (WS) include alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins.





Chemical structure of withaolide **structure of withanolide.A**

3.7 EXCIPIENT PROFILE:

ETHANOL

Chemical names: Ethanol, Ethyl alcohol, Alcohol

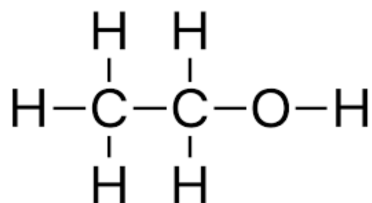
Molecular Formula: CH_3CH_2OH; C_2H_6O

Molecular Weight: 46.069 g/mol

Description:

Ethanol is a clear, colorless liquid rapidly absorbed from the gastrointestinal tract and distributed throughout the body. It has bactericidal activity and is used often as a topical disinfectant. It is widely used as a solvent and preservative in pharmaceutical preparations as well as serving as the primary ingredient in alcoholic beverages. Indeed, ethanol has widespread use as a solvent of substances intended for human contact or consumption, including scents, flavorings, colorings, and medicines(56).

Structure formula:



Applications of Ethanol in Pharmaceutical Formulation and Technology:

- **E**thanol can be found as an active ingredient in oral, parenteral, and topical (including inhalational) prescription and nonprescription drug products.
- Although it is primarily used because of its solvent properties to help solubilize many drugs, it also possesses several concentration-dependent pharmacological actions, including sedative, carminative, cooling, antipyretic, rubefacient, cleansing, and antiseptic properties.

- Concentrations of 40% or more may be found in some oral preparations, thus resulting in patients consuming a significant amount of alcohol during the course of the day[57].

PROPYLENE GLYCOL:

Chemical names: Propylene glycol, 1,2 propanediol, Propane 1,2- diol.

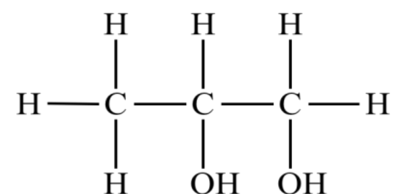
Molecular formula: $C_3H_8O_2$

Molecular weight: 76.095g/mol

Description:

Propylene glycol is a clear, colorless, viscous organic solvent and diluent used in pharmaceutical preparations. Propylene glycol is used as a solvent for intravenous, oral, and topical pharmaceutical preparations. It is generally considered safe. However in large doses it can be toxic, especially if given over a short period of time.

Structure formula:



Applications of propylene glycol in pharmaceutical formulation and technology:

- Propylene glycol is used as a humectant, solvent and preservative in food and for tobacco products. It is also one of the major ingredients along with Vegetable Glycerin(59).
- Propylene glycol is used as a solvent in many pharmaceuticals including oral, injectable and topical formulations.
- Propylene glycol is used in veterinary medicine as an oral treatment for hyperketonaemia in ruminants.

SOYALECITHIN

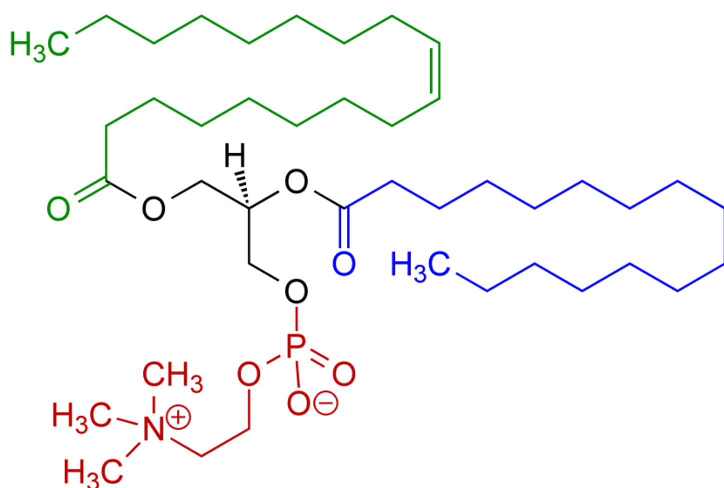
Chemical names: 1-Palmitoyl-2-linoleoylphosphatidylcholine

Molecular formula: $C_{42}H_{80}NO_8P$

Molecular weight: 758.1 g/mo

Description: Lecithin is used to stabilize emulsions. Lecithin is present in all living cells and is a significant constituent of nerve and brain cells. Commercial lecithin primarily comes from soybean oil. The FDA considers soya lecithin as generally regarded as safe as a food product when consumed in normal amounts(60).

structure of soya lecithin:



Applications of soya lecithin in pharmaceutical formulation and technology:

- Phospholipids are used in pharmaceutical technology as wetting agents, emulsifiers, and builder or components of mesophases like liposomes, micelles, mixed micelles, cubosomes, etc.
- These functional properties are used in many formulation types, like suspensions, various types of emulsions, mixed micelles, solid dispersions, drug–phospholipid complexes, etc.

- Due to their physiological role, phospholipids possess a very low toxicity profile and can be used for any route of administration.

3.8 EXPERIMENTAL METHODOLOGY:

Materials:

Herbal Drug: withania somnifera

Reagents and chemicals:

Soxhlet apparatus

Wagner's reagent

Mayer's reagent

Hager's reagent

Fecl3 solution

Sodium hydroxide solution

Dilute hydrochloric acid

Sulphuric acid

Ammonia

Chloroform

METHODOLOGY:

Extraction procedure:

Roots of withania somnifera was taken from ayurvedic medical store. Firstly, dried sample was extracted with solvent of ethyl acetate, n hexane and ethanol in the ratio of 50:50:200 at 40°C for

10 hours in soxhlet apparatus. The residue was dried under reduced pressure by using a rotary vacuum evaporator.

3.9 Preliminary Phytochemical Studies Of Root Extract Of Withania Somnifera:

Qualitative tests were used to analyse the phytochemical compounds in the solvent free extract of roots of withania somnifera.

A. Test for carbohydrates

There are some tests performed for carbohydrates.

a) Molisch's test: Sample of plant extract was taken in a test tube. Then 20% alcoholic solution and concentrated sulphuric acid, which is freshly prepared is added in to test tube along the sides. This test develops reddish violet and purple colour at junction between two liquids if carbohydrates present in the sample extracts[(61)].

b**) Benedict's test**: Take a test tube, which contain small amount of plant extracts sample. In a test tube added small quantity of benedict's solution and mix properly. Then boil this sample mixture for two minutes and cool it. If carbohydrates present in the sample, it forms red precipitate.

c) Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. If carbohydrates present in the sample extracts, it forms red precipitate of copper oxide.

B. Test for alkaloids

a) Dragendorff's test: Take a few mg of extracts sample and dissolve in 5ml water. Then 2 M hydrochloric acid added until an acid reaction developed. In this mixture, 1ml of dragendorff's reagent (potassium bismuth iodine solutions) was added. If alkaloids present in sample extracts, it forms orange red precipitate.

b) Wagner's test: Acidify the plant extract sample with hydrochloric acid (1.5% v/v) and add a few drop of Wagner's reagent (iodine potassium iodide solution) in the test tube. It forms reddish brown precipitates which indicate the presence of alkaloids[(62)].

c) Mayer's test: 2ml of plant extracts sample was taken and 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodine solution) in the test tube. If alkaloids present in the sample, it form dull white precipitate.

C. Test for glycosides

a) Legal's test: Take a extracts sample and dissolved in pyridine then add sodium nitroprusside solution. Make this solution completely alkaline. Presence of glycosides produce pink red colour.

b) Baljet's test: Take a plant extracts sample in the test tube and add sodium picrate solution. Presence of glycosides produce yellow to orange colour [63].

c) Borntrager's test: The test solution of plant extract was added in few ml of dilute sulphuric acid solution. This solution was filtered. Then Chloroform and ether was added in to filtrate and shaken well. In this solution ammonia was added and separated the organic layer. Organic layer shows pink, red or violet colour due to the presence of glycosides.

D. Test of saponins

a)1ml of alcoholic sample extract was taken and diluted with 20ml of distilled water. This solution was shaked for 15 min in graduated cylinder. If saponins present [64] in the extracts, it generate foam layer of 1cm.

E. Test for flavonoids

a) Shinoda test: Taken the alcoholic sample extract in the test tube and 5-10 drops of hydrochloric acid added in the sample. Then small pieces of magnesium added in tubes. Reddish pink or brown colour was indicated the presence of flavonoids.

b) Alkaline reagent test: Plant extracts sample was mixed with 2ml of 2% NaOH solution. It produced yellow colour. In this solution, 2 drops of diluted acids was added. If flavonoids present in the extracts, yellow colour changed into colorless[65].

F.Test for tannins

a)Take the sample of plant extracts in the test tube and added ferric chloride solution. If tannin present in the sample, dark blue or greenish black colour appeared[67].

b)Take the sample extracts and add potassium cyanide. It produce deep red colour, which indicate the presence of tannins.

c)Potassium dichromate was added in to sample extracts. Yellow precipitate was formed indicate the presence of tannins.

G. Test for protein and amino acid

a)Biuret's test: Take 2-3 ml of sample extract and add 1 ml sodium hydroxide solutions (40%) and 2 drops of copper sulphate solution (1%) and mix properly. Presence of proteins shows a pinkish - violet and purple - violet colour(68).

b)Ninhydrin's test: Plant extracts sample mixed with freshly prepared 2 drops of 0.2% ninhydrin solution and heated to boiling for 1-2 min and allowed cooling. Blue colour appearance indicates the presence of amino acids, proteins, peptides[(69)].

c) Xanthoprotein test: Extracts sample was taken in test tube and added conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. 20% sodium hydroxide solution added in excess, which produce orange colour that indicate the presence of amino acids[(70)].

H. Test of fats or fixed oils

a) Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then 10% sodium hydroxide solution was added. Blue colour appears in the solution, which shows the presence of glycerin[(71)].

b) Saponification: plant extracts was taken and mixed with 2% sodium carbonate solution. Shaked vigorously and boiled. A clean soapy solution was formed cooled and few drops of conc. HCl was added and observed that fatty separate out and float up [(72)].

3.10 Antimicrobial Activity Of Extracts:

Activities of the extract against 3 clinical pathogens were observed using Kirby Bauer's well method. Pathogens were plated on nutrient agar medium and 100 µl of the extract was placed in the wells, incubated for 24-48 h at 32˚C. The plates were checked for zone of inhibition (diameter) after incubation is over, interpret the results. The clinical pathogens used were Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli . They were obtained from Microbial Biotechnology Laboratory [73].

3.11 Thin Layer Chromatography (TLC):

For TLC determination of the extract, silica gel plated glass slides were used with different solvent systems like acetonitrile, chloroform and methanol ratios ,(1:8:1). Later the plates were observed under UV with 5% potassium hydroxide as the detection reagent[74]

3.12 Preparation of ethosomes from the root extract of withania somnifera by cold and hot method:

Cold Method:

Materials

Root extract of Withania somnifera

Soya lecithin (HIMEDIA Laboratories Pvt. Limited, Mumbai.),

Ethanol (SD fine-chem. Limited, Mumbai.),

Propylene glycol (SD fine-chem. Limited, Mumbai.),

Methanol (SD fine-chem. Limited, Mumbai.),

Potassium Dihydrogen phosphate (SD fine-chem Limited, Mumbai.),

Sodium hydroxide (SD fine-chem. Limited, Mumbai.),

Double distilled water[75].

General procedure:

Required amount of soya lecithin and drug was taken and few ml of ethanol was added and then the above mixture was kept for magnetic stirring. While string small quantity of propylene glycol was added at temperature 30^0c and speed maintained at 700rpm for 20mim. Another phase was prepared by taking distilled water in a beaker and the aqueous phase was heated at 30^0c. Then the aqueous phase was added to the organic phase . The above mixture was stirred for 1hr which leads to the formation of ethosomes(76).

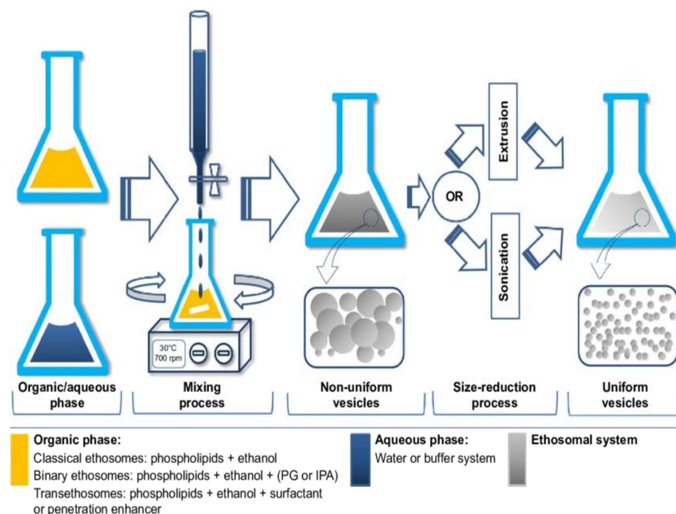


Figure 3.12.1:COLD METHOD

Table 3.13: List and composition of formulations prepared by Cold method

Formulation code	Drug (withania somnifera) in mg	Lipid soya lecithin (Mg)	Ethanol	Propylene Glycol and IPA in ml
E1	10	10	4	2
E2	10	20	4	2
E3	10	30	4	2
E4	10	40	4	2
E5	10	50	4	2
E6	10	60	4	2

Hot Method:

Materials

Root extract of withania somnifera

Soya lecithin (HIMEDIA Laboratories Pvt. Limited, Mumbai.),

Ethanol (SD fine-chem. Limited, Mumbai.),

Propylene glycol (SD fine-chem. Limited, Mumbai.),

Methanol (SD fine-chem. Limited, Mumbai.),

Potassium Dihydrogen phosphate (SD fine-chem Limited, Mumbai.),

Sodium hydroxide (SD fine-chem. Limited, Mumbai.),

Double distilled water.

General procedure:

This method provides a means of making ethosomes. In this method, phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach40°C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties[77].

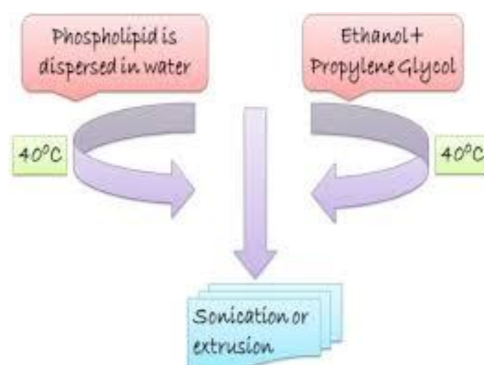


Table 3.14: List and composition of formulations prepared by Hot method:

Formulation code	Drug (withania somnifera) in mg	Lipid soya lecithin (Mg)	Ethanol	Propylene Glycol and IPA in ml
E1	10	10	4	2
E2	10	20	4	2
E3	10	30	4	2
E4	10	40	4	2
E5	10	50	4	2
E6	10	60	4	2

3.15: Characterization And Evaluation Of The Ethosomes:

Particle size measurement

It is done by Partical analyzer (HORIBA SZ-100 series).Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy, is a common technique for measuring the size of particles in the sub-micron range. It measures Brownian motion of particles suspended within a liquid, through changes in the intensity of light scattered from particles through time. Consequently, the slower the motion the larger the particle will be, since smaller particles are more affected by interactions with the solvent. Considering this motion, and the temperature and viscosity of the sample throughout the analysis, DLS can calculate the hydrodynamic diameter of the particle [(78)].

Zeta potential:

The zeta potential is a physical property which is exhibited by all particles in suspension. It is an important parameter in understanding the electric double layer repulsion and it can be measured by phase analysis light scattering. When an electric field is applied across an electrolyte, charged particles in suspension are attracted towards the electrode of opposite charge while viscous forces acting on the particles tend to oppose the movement. When equilibrium is reached, the particles move with constant velocity, also known as electrophoretic mobility, and the zeta potential can be measured [(79)]. The magnitude of the zeta potential gives an indication of the potential stability of the system. If the modulus of the zeta potential is large, the particles in suspension will tend to repel each other. Hence, there will be no tendency to agglomerate. Contrastingly, when zeta potential values are low, it means that there will be no force to prevent the particles coming together and agglomerate [(80)].

Scanning electron microscopy

Scanning electron microscopy (SEM) is based on the incidence of a beam of accelerated electrons on the sample. These accelerated electrons interact with the sample, exciting its atoms which emit secondary electrons. According to the angle between the primary beam and the surface of the sample, it is possible to detect and analyze the surface topography [(81)].

3.16: EVALUATION OF ETHOSOMES:

The obtained Ethosomes by the above technique were evaluated for

1. Drug content

2. Entrapment efficiency

3. In vitro diffusion studies

1.Optical Microscopy:

Morphology was determined for all the prepared invasomal formulations using optical microscopy (-3700N, Hitachi, Japan). The photographic pictures of the preparation was obtained from the microscope by using a SLR camera.

2.Drug Content:

The prepared ethosomal formulation taken and from the suspension 1ml was taken and transferred into the 10ml volumetric flask and the volume was made with methanol to disrupt methanol vesicles by thoroughly shaking up to 10 minutes and from this 0.1 ml of solution was taken and suitable dilutions were made and the concentration of drug was analyzed using UV spectrophotometer at 250nm[81].

3.Entrapment efficiency :

The Ethosomal formulations were examined for entrapment efficiency. Entrapment efficiency was conducted by taking 1ml of suspension and diluted with 9ml of pH 7.4 phosphate buffer in 10 ml volumetric flask. The nanoparticles suspension is ultra-centrifuged at 17240 rpm and temperature of -4 ℃ for 40 minutes. Then the supernatant liquid was taken out to check for the absorbance by using UV with the necessary dilutions. The entrapment efficiency can be expressed as follows:

$$\%\text{Entrapment efficiency} = \frac{\text{Total drug added} - \text{un entrapped drug}}{\text{Total drug added}} \times 100$$

4. **Invitro Drug diffusion studies: -** Drug diffusion studies were performed by using Franz diffusion cell. A known amount of ethosomal suspension was separately pippeted out and transferred to the donor compartment and 50 ml of the pH 7.4 Phosphate buffer was taken in the receptor compartment. The temperature and stirring speed was adjusted to 37°C and 100 rpm, respectively. At predetermined time intervals of 0.5, 1, 2,3, 4,5, 6,7, 8,9, 10,11, 12 hours alliquotes of 1ml of samples were withdrawn and the same volume was replaced with fresh medium to maintain sink conditions. The samples were further analyzed using UV Spectrophotometer at 250nm[83].

5.Determination of size distribution, poly dispersity index (PDI) and zeta potential:

The prepared invasomes were dispersed in double distilled water and sonicated for 3 hrs. The resultant dispersion was diluted and observed for particle size and zeta values by using Malvern Zeta Sizer.

CHAPTER- 4RESULT

4.RESULTS

4.1 EXTRACTION:

The dried root powder is extracted using solvents such as ethanol, ethyl acetate soxhlet apparatus**.**

Fig No. 4.1: Dried withania somnifera root extract

TABLE NO. 4.2: Determination of phytochemical constituents of withania somnifera.

PHYTOCHEMICALS	TEST	OBSERVATION	INFERENCE
Carbohydrates	a)Molisch s test b)Benedicts test c. Fehling's test	Appearance of purple ring indicates presence of carbohydrates.	It confers the absence of carbohydrates
Alkaloids	Dragendroffs test Wagners test Mayers test	Appearance of reddish brown colored precipitate	It confers the presence of alkaliods

Glycosides	a) Legals test b) Killer killian test	Appearance of blue colour indicates presence of glycosides	It confers the presence of glycosides
Saponins	Saponins test	White ppt formed	It confers the presence of saponins
Flavonoids	a) Shinoda test b) Alkaline re- agent test	Formation of light pink color Brown colour was indicated	It confers the presence of flavonoids
Tannins	Tannins test	1.When ferric chloride solution was added green colour developed. 2.When pottasium dichromate was added yellow ppt formed	It confers the presence of tannins
Proteins and amino acids		1.When copper sulphate was added pinkish violet colour developed 2.When ninhydrin reagent was added blue colour was developed	It confers the presence of proteins and aminoacids

Sterols	libermann’s test	Formation of brown precipitate indicate the presence of sterols.	It confers presence of sterols.
	Salkowski test	Appearance of red colour indicates presence of sterols	presence of sterols

From the above table, the presence of, alkaloids, sterols, saponins, flavonoids, Tannins, proteins and amino acids was observed.

4.3 Thin Layer Chromatography:

Withania somnifera was subjected to TLC studies by taking withanolide as standard. To confirm the presence of major compound , mobile phase taken as chloroform, methanol and acetonitrile in a ratio such as 9:1:1 Rf value was found to be 0.88 for standard withanolide and the root extract was found to be 0.99 indicated the presence of steroids as major compound.

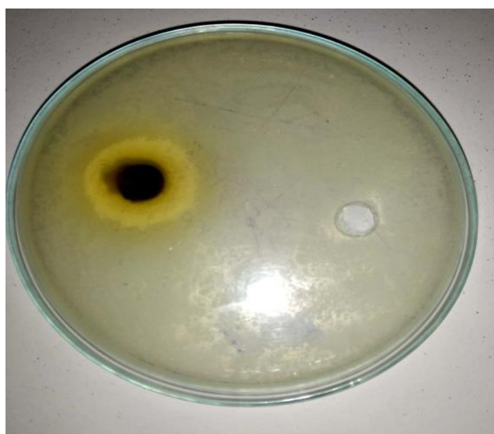
RS (EXTRACT OF WITHANIA SOMNIFERA)

Fig 4.3 Tlc study of root extract of withania somnifera

4.4 Determination of Antimicrobial Activity:

All the pathogenic micro-organism tested . The ethanolic extract of roots of withania somnifera was highly effective against staphylococcus aureus with zone of inhibition 30 mm, pseudomonas argenuosa with a zone of inhibition 31mm, E coli with a zone of inhibition 26 mm respectively. It has been compared with streptomycin disc. The zone of inhibition for this disc was found to be 25 mm. So, the withania somnifera extract was exhibiting good anti-microbial activity than streptomycin disc.

Figure 4.4.1 Anti-microbial activity of withania somnifera against staphylococcus



4.4.2 Anti-microbial activity of withania somnifera against Pseudomonas

4.4.3 Anti-microbial activity of withania somnifera against Ecoli

4.5 EVALUATION PARAMETERS:

4.6 RESULTS AND DISCUSSION OF ETHOSOMAL FORMULATION OF WITHANIA SOMNIFERA BY COLD METHOD:

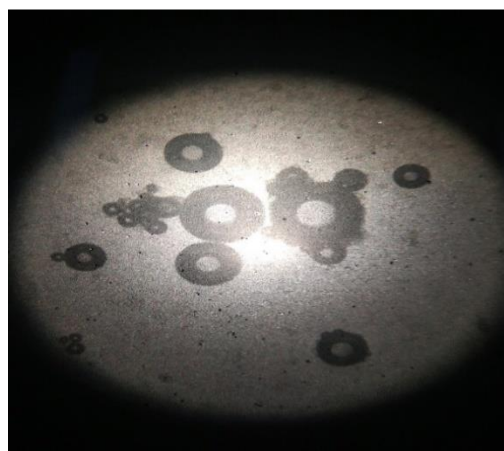
Determination of Effect of Lipid Concentration Upon Formulation of Ethosomes:

Six formulations were prepared by altering drug to lipid ratio i.e. by increasing lipid concentration from10mg to 60mg. All the obtained formulations has been evaluated for drug content, entrapment efficiency, invitro diffusion studies, particle size and zeta potential.

Determination of vesicle size of Withania somnifera loaded ethosomes by Optical Microscopy:

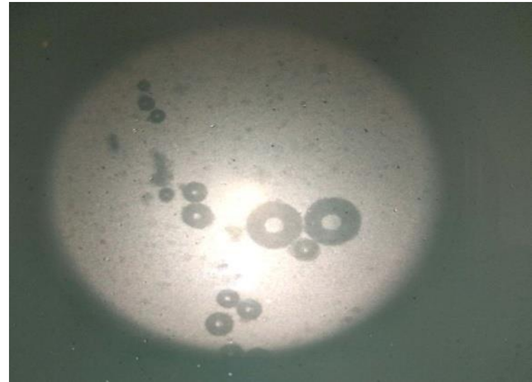
The formation of vesicles were confirmed by optical microscopy. The ethosomal formulation of Withania somnifera when observed under projection microscope showed multi-lamellar vesicles.

(1:1)

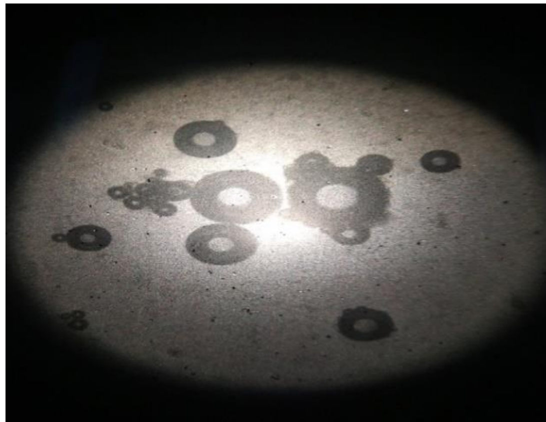


(1:2)

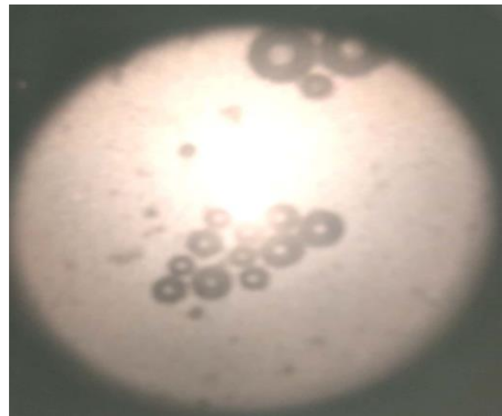
(1:3)



(1:4)



(1:5)



(1:6)

Figure 4.6.1: Projection microscopic images of ethosomes prepared by cold method

Vesicle Size Distribution:

The prepared six formulations were characterized for vesicle size using Zetasizer (Malvern Instruments Ltd.). The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

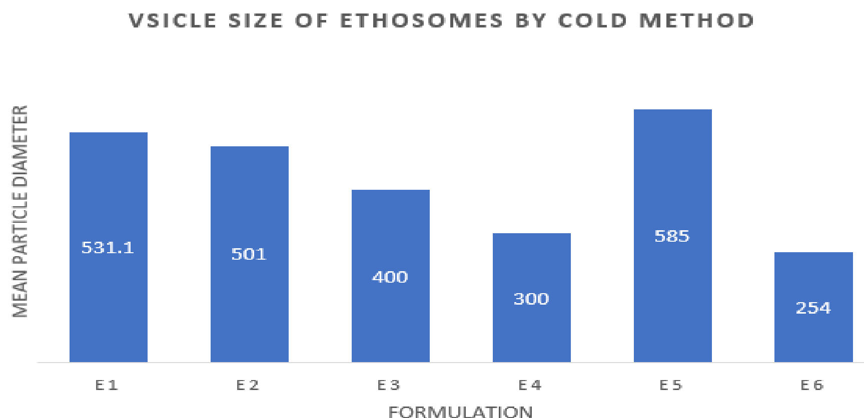


Figure 4.6.2: Comparison of mean vesicle diameter of withania somnifera loaded ethosomes by cold method.

All six formulations were in Nano size range. The mean vesicle diameter of E1, E2, E3, E4, E5 and E6 formulations was found to be 531.1nm, 501nm, 400nm, 300nm, 585nm and 254.6nm respectively. Out of the six formulations the E6 formulation (1:6) was found to be the best formulation because of its small mean vesicle diameter of 254.6nm.

Sample Details

Sample Name: ETHOSOMES C (1;6)

SOP Name: 12-5.sop

General Notes:

File Name:	RBVRR college.dts	Dispersant Name:	Water
Record Number:	12	Dispersant RI:	1.330
Material RI:	1.59	Viscosity (cP):	0.8872
Material Absorbtion:	0.010	Measurement Date and Time:	30 September 2020 11:46:30

System

Temperature (°C):	25.0	Duration Used (s):	70
Count Rate (kcps):	183.9	Measurement Position (mm):	4.65
Cell Description:	Glass cuvette with square ape...	Attenuator:	11

Results

D(0.1): 130 nm D(0.5): 3800 nm D(0.9): 5940 nm

			Diam. (nm)	% Volume	Width (nm)
Z-Average (d.nm):	254.6	Peak 1:	140.0	20.5	44.25
PdI:	0.279	Peak 2:	474.0	23.8	228.4
Intercept:	0.950	Peak 3:	4948	55.6	882.2
Result quality :	Good				

Size Distribution by Volume

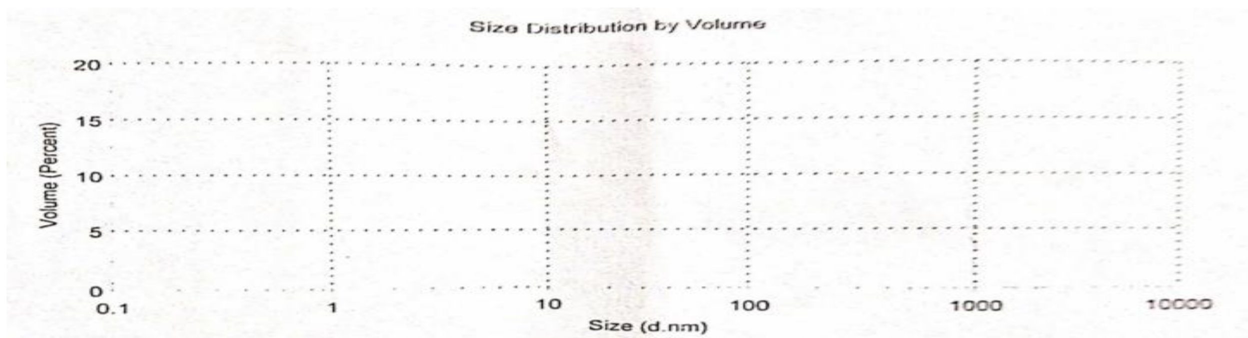


Figure 4.6.3: Vesicle size distribution report of optimized E6 formulation of Withania somnifera loaded ethosomes by cold method.

Zeta Potential:

The prepared six formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

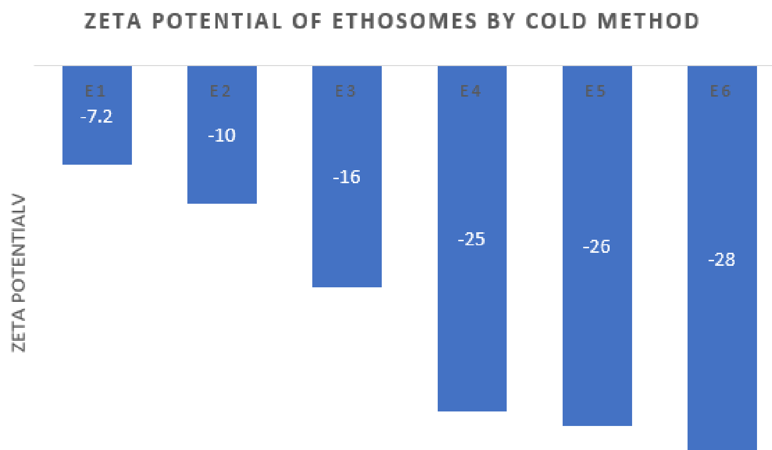
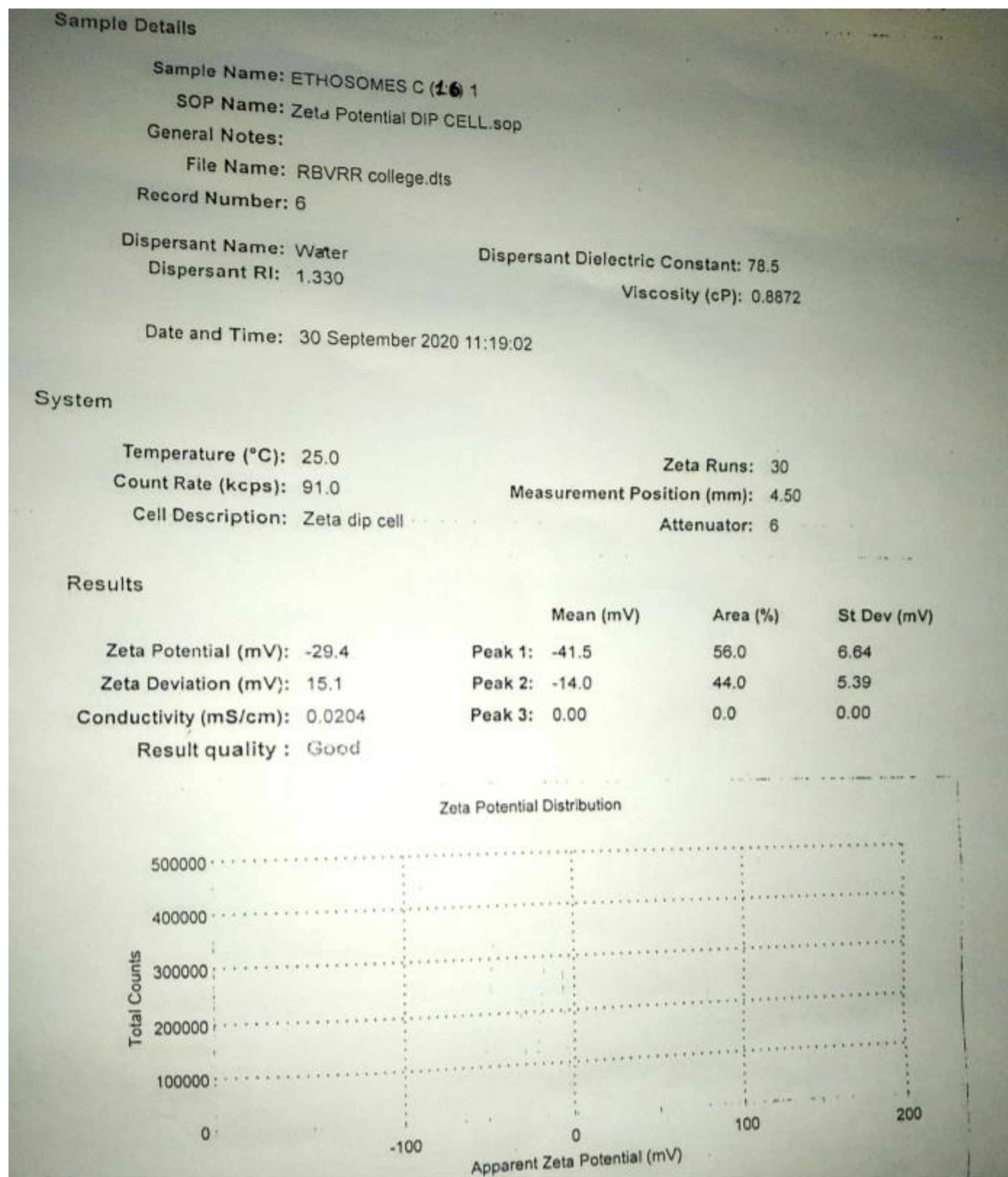


Figure 4.6.4: Comparison of zeta potential values of six formulations of withania somnifera loaded ethosomes by cold method.

From the results all the formulations were found to be stable. The zeta potential values of E1- E6 formulations was found to be -7.2mV, -10mV, -16 mV, -25mV, -26mV and -29.4mV respectively. Out of the six formulations the E6 formulation (1: 6) was found to be stable formulation with highest zeta potential value of -29.4mV.



Sample Details

Sample Name: ETHOSOMES C (16) 1
SOP Name: Zeta Potential DIP CELL.sop
General Notes:
File Name: RBVRR college.dts
Record Number: 6

Dispersant Name: Water
Dispersant RI: 1.330
Dispersant Dielectric Constant: 78.5
Viscosity (cP): 0.8872

Date and Time: 30 September 2020 11:19:02

System

Temperature (°C): 25.0
Count Rate (kcps): 91.0
Cell Description: Zeta dip cell
Zeta Runs: 30
Measurement Position (mm): 4.50
Attenuator: 6

Results

Zeta Potential (mV): -29.4
Zeta Deviation (mV): 15.1
Conductivity (mS/cm): 0.0204
Result quality : Good

	Mean (mV)	Area (%)	St Dev (mV)
Peak 1:	-41.5	56.0	6.64
Peak 2:	-14.0	44.0	5.39
Peak 3:	0.00	0.0	0.00

Figure 4.6.5: Zeta potential report of optimized E6 formulation of withania somnifera loaded ethosomes by cold method.

Drug Content:

The prepared six formulations were evaluated for drug content.

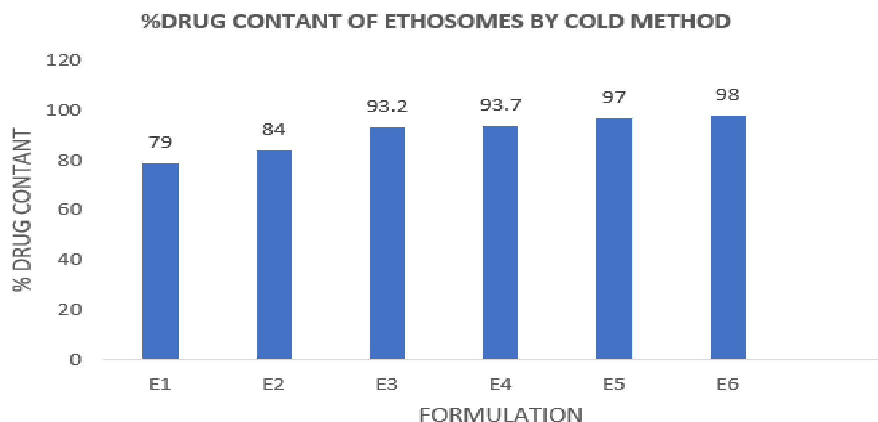


Figure 4.6.6: Comparison of drug content among the six formulations of withania somnifera loaded ethosomes by cold method.

Drug content of E1, E2, E3, E4, E5 and E6 was found to be 79, 84, 93.2, 93.7 ,97 and 98 respectively. Out of the six formulations the E6 formulation containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest drug content of 98%.

Entrapment Efficiency:

The prepared six formulations were evaluated for entrapment efficiency.

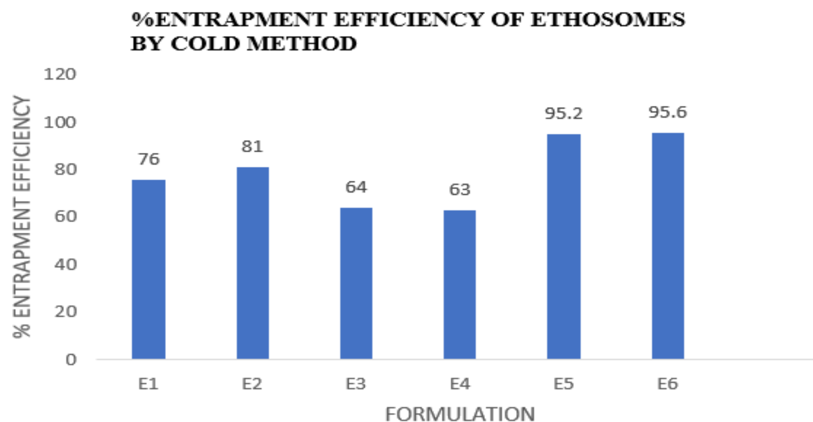


Figure 4.6.7: Comparison of entrapment efficiency among the six formulations of withania somnifera loaded ethosomes by cold method.

Entrapment efficiency of E1, E2, E3, E4, E5 and E6 was found to be 76, 81, 64, 63, 95.2 and 95.6% respectively. Out of the six formulations the E6 formulation containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest entrapment efficiency of 95.6%.

In Vitro Diffusion Studies:

The prepared six formulations were evaluated for invitro drug diffusion studies.

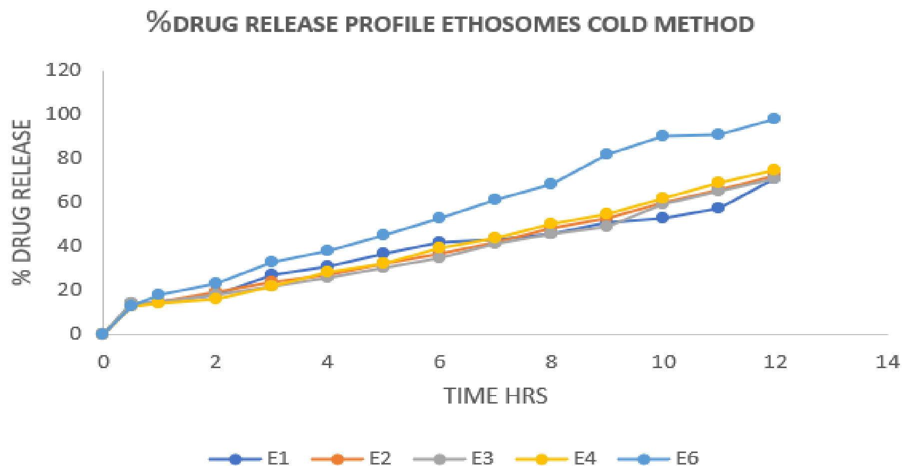


Figure 4.6.8: Comparison of invitro drug diffusion studies among the six formulations of withania somnifera loaded ethosomes by cold method.

The prepared six formulations were evaluated for invitro drug diffusion studies using Franz diffusion cell. In vitro drug diffusion studies were conducted for a time period of 12 hrs. The percentage of drug release of E1, E2, E3, E4, E5 and E6 formulations was found to be 71%, 75%, 72%, 71% and 98% respectively. E6 formulation containing 1:6 ratio of drug to lipid was considered the better formulation because of its highest drug release of 98%.

4.7 RESULTS AND DISCUSSION OF ETHOSOMAL FORMULATION OF WITHANIA SOMNIFERA BY HOT METHOD:

Determination of Effect of Lipid Concentration Upon Formulation of Ethosomes:

Six formulations were prepared by altering drug to lipid ratio i.e. by increasing lipid concentration from10mg to 60mg. All the obtained formulations has been evaluated for drug content, entrapment efficiency, invitro diffusion studies, particle size and zeta potential.

Determination of vesicle size of Withania somnifera loaded ethosomes by Optical Microscopy:

The formation of vesicles were confirmed by optical microscopy. The ethosomal formulation of Withania somnifera when observed under projection microscope showed multi-lamellar vesicles.

(1:1)

(1:2)

Figure4.7.1: Projection microscopic images of ethosomes prepared by hot method

Vesicle Size Distribution:

The prepared six formulations were characterized for vesicle size using Zetasizer (Malvern Instruments Ltd.). The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

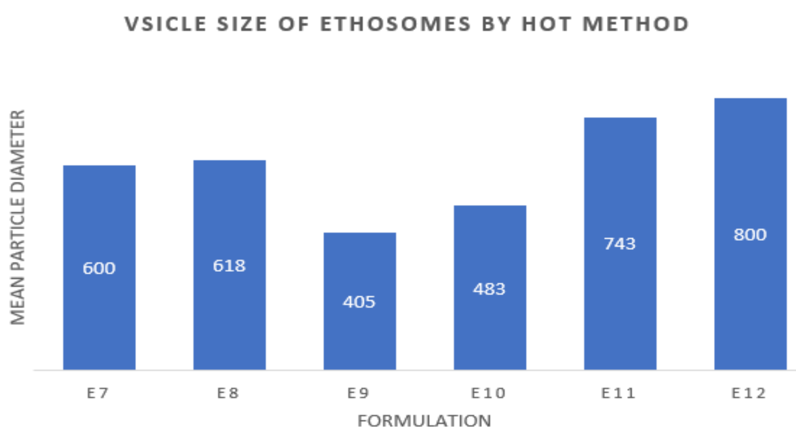
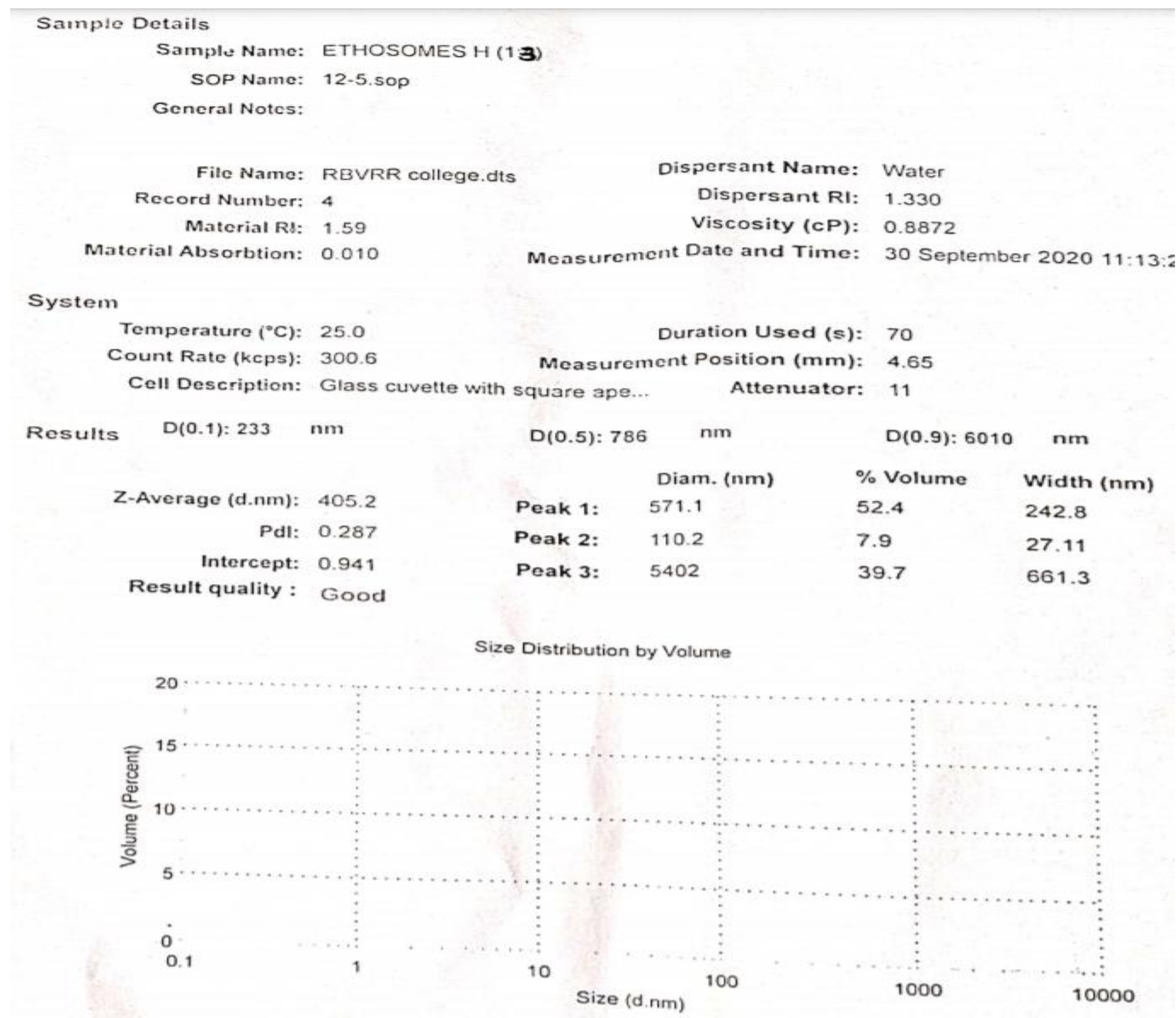


Figure 4.7.2: Comparison of mean vesicle diameter of withania somnifera loaded ethosomes by hot method.

All six formulations were in Nano size range. The mean vesicle diameter of E7, E8, E9, E10, E11 and E12 formulations was found to be 600nm, 618nm, 405.8nm, 483nm, 743nm and 800nm

respectively. Out of the six formulations the E9 formulation (1:3) was found to be the best formulation because of its small mean vesicle diameter of 405.8nm.



Sample Details

Sample Name: ETHOSOMES H (13)

SOP Name: 12-5.sop

General Notes:

File Name: RBVRR college.dts

Record Number: 4

Material RI: 1.59

Material Absorbtion: 0.010

Dispersant Name: Water

Dispersant RI: 1.330

Viscosity (cP): 0.8872

Measurement Date and Time: 30 September 2020 11:13:2

System

Temperature (°C): 25.0

Count Rate (kcps): 300.6

Cell Description: Glass cuvette with square ape...

Duration Used (s): 70

Measurement Position (mm): 4.65

Attenuator: 11

Results D(0.1): 233 nm D(0.5): 786 nm D(0.9): 6010 nm

Z-Average (d.nm): 405.2

PdI: 0.287

Intercept: 0.941

Result quality : Good

	Diam. (nm)	% Volume	Width (nm)
Peak 1:	571.1	52.4	242.8
Peak 2:	110.2	7.9	27.11
Peak 3:	5402	39.7	661.3

Figure 4.7.3: Vesicle size distribution report of optimized E9 formulation of Withania somnifera loaded ethosomes by hot method.

Zeta Potential:

The prepared six formulations were characterized for zeta potential value in order to know the stability of the formulations. The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

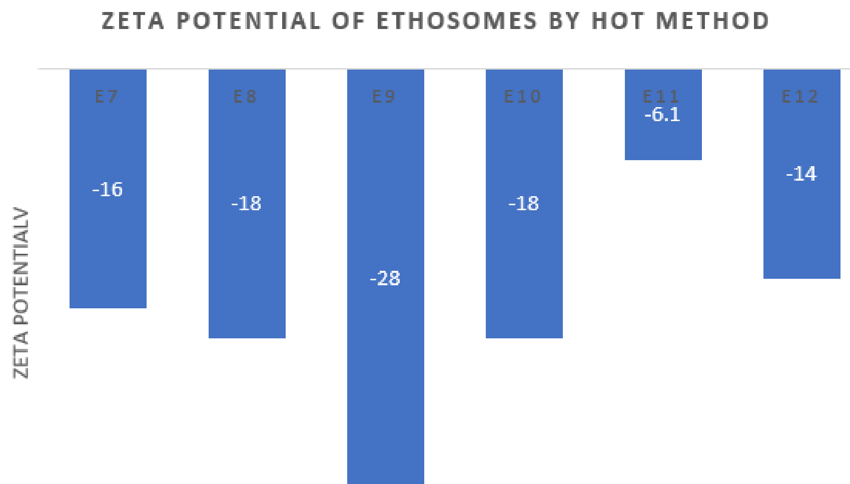
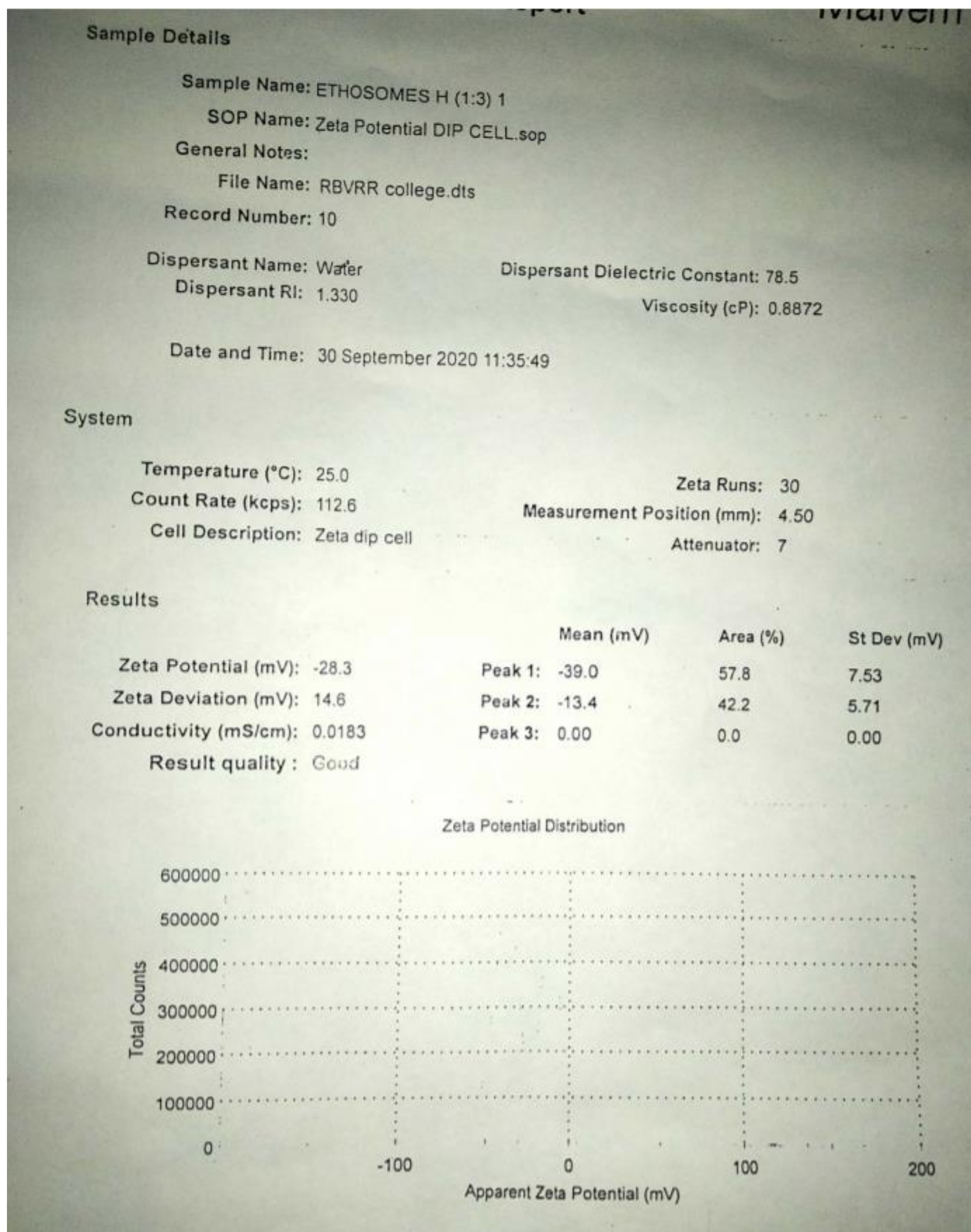


Figure 4.7.4: Comparison of zeta potential values of six formulations of withania somnifera loaded ethosomes by hot method.

From the results all the formulations were found to be stable. The zeta potential values of E7- E12 formulations was found to be between -16mV, -18mV, -28mV, -18mV, -6.1mV and -14mV respectively. Out of the six formulations the E9 formulation (1:3) was found to be stable formulation with highest zeta potential value of -28mV.



Malvern

Sample Details

Sample Name: ETHOSOMES H (1:3) 1
SOP Name: Zeta Potential DIP CELL.sop
General Notes:
File Name: RBVRR college.dts
Record Number: 10

Dispersant Name: Water
Dispersant RI: 1.330
Dispersant Dielectric Constant: 78.5
Viscosity (cP): 0.8872

Date and Time: 30 September 2020 11:35:49

System

Temperature (°C): 25.0
Count Rate (kcps): 112.6
Cell Description: Zeta dip cell
Zeta Runs: 30
Measurement Position (mm): 4.50
Attenuator: 7

Results

Zeta Potential (mV): -28.3
Zeta Deviation (mV): 14.6
Conductivity (mS/cm): 0.0183
Result quality : Good

	Mean (mV)	Area (%)	St Dev (mV)
Peak 1:	-39.0	57.8	7.53
Peak 2:	-13.4	42.2	5.71
Peak 3:	0.00	0.0	0.00

Figure 4.7.5: Zeta potential report of optimized E9 formulation of withania somnifera loaded ethosomes by hot method.

Drug Content:

The prepared six formulations were evaluated for drug content.

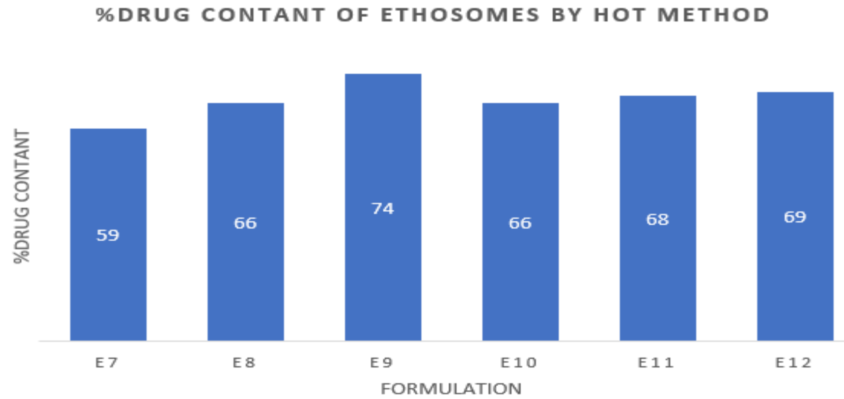


Figure 4.7.6: Comparison of drug content among the six formulations of withania somnifera loaded ethosomes by hot method.

Drug content of E7, E8, E9, E10, E11and E12 was found to be 59%, 66%, 74%, 66%, 68% and 99% respectively. Out of the six formulations the E9 formulation containing 1:3 ratio of drug to lipid was considered the best formulation because of its highest drug content of 74%.

Entrapment Efficiency:

The prepared six formulations were evaluated for entrapment efficiency.

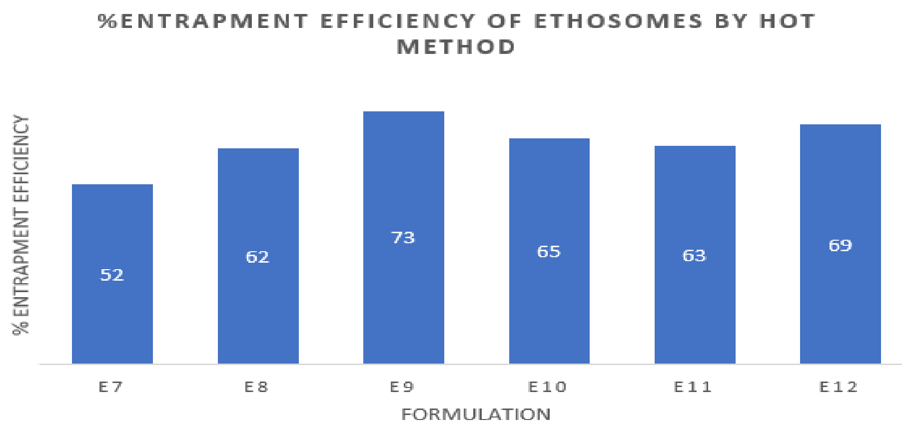


Figure 4.7.7: Comparison of entrapment efficiency among the six formulations of withania somnifera loaded ethosomes by hot method.

Entrapment efficiency of E7, E8, E9, E10, E11 and E12 was found to be 52%, 62%, 73%, 65%, 63% and 69% respectively. Out of the six formulations the E9 formulation containing 1:3 ratio of drug to lipid was considered the best formulation because of its highest entrapment efficiency of 73%.

In Vitro Diffusion Studies:

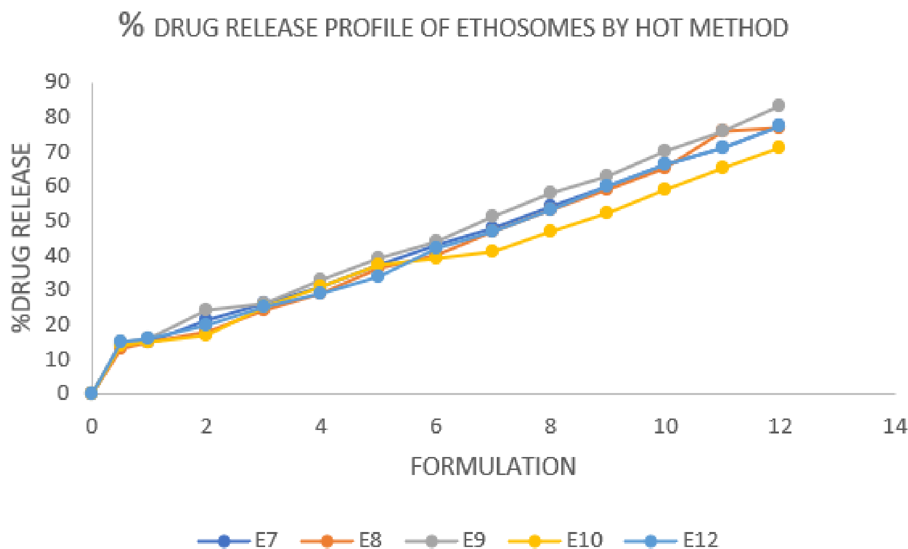


Figure 4.7.8: Comparison of invitro drug diffusion studies among the six formulations of withania somnifera loaded ethosomes by hot method.

The prepared six formulations were evaluated for invitro drug diffusion studies using Franz diffusion cell. In vitro drug diffusion studies were conducted for a time period of 12 hrs. The percentage of drug release of E7, E8, E9, E10, E11 and E12 formulations was found to be 77.5%, 77%, 83%, 78% and 77% respectively. E9 formulation containing 1:3 ratio of drug to lipid was considered the better formulation because of its highest drug release of 83%.

4.8 COMPARATIVE STUDY AMONG THE BEST FORMULATIONS OF WITHANIA SOMNIFERA LOADED ETHOSOMES BY HOT AND COLD METHOD:

Withania somnifera loaded ethosomes were prepared by cold and hot method by varying drug to lipid ratio. Total twelve formulations were prepared and all the formulations were evaluated for drug content, entrapment efficiency, zeta potential, mean vesicle size and invitro drug diffusion studies.

On comparison, ethosomal formulation of Withania somnifera prepared by cold method containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest drug content of 98%, entrapment efficiency of 95.6%, particle size of 254.6 nm, zeta potential of -29.4mV and drug release of 98% in a time period of 12 hrs.

On comparison, ethosomal formulation of Withania somnifera prepared by hot method containing 1:3 ratio of drug to lipid was considered the best formulation because of its highest drug content of 74%, entrapment efficiency of 73%, particle size of 405.2 nm, zeta potential of -28.3mV and drug release of 83% in a time period of 12 hrs.

Vesicle Size Distribution:

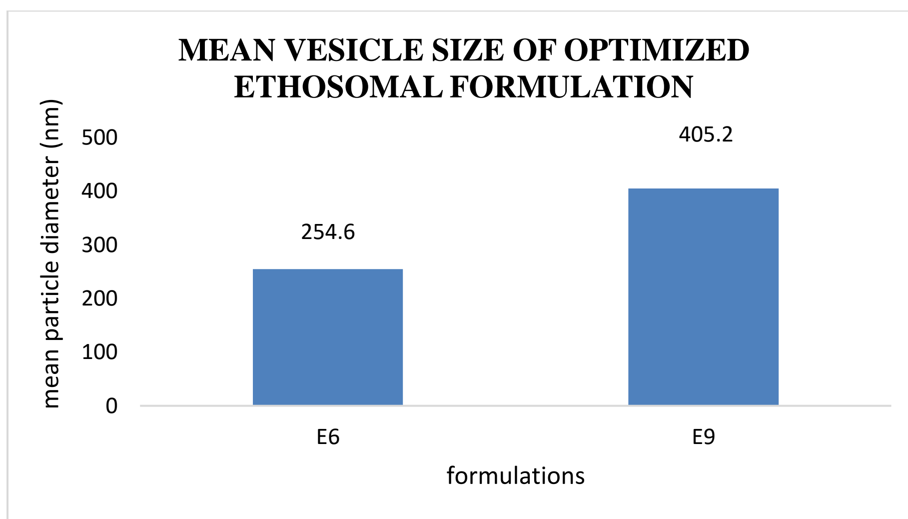


Figure 4.8.1: vesicle size diameter of optimized ethosomal formulations

The mean vesicle diameter of the two best formulations were compared. Out of the two formulations the E6 formulation containing 1:6 ratio of drug to lipid was found to be the best formulation because of its small mean vesicle diameter of 254.6nm.

Zeta potential

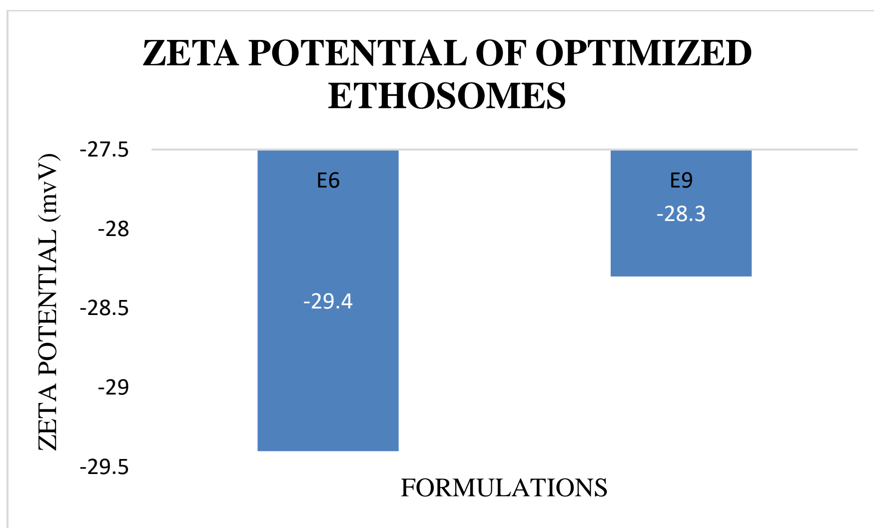


Figure 4.8.2: zeta potential values of optimized ethosomal formulations

The zeta potential values of the two best formulations were compared. Out of the two formulations the E6 formulation containing 1:6 ratio of drug to lipid was found to be stable formulation with highest zeta potential value of -28.0mV.

Drug content

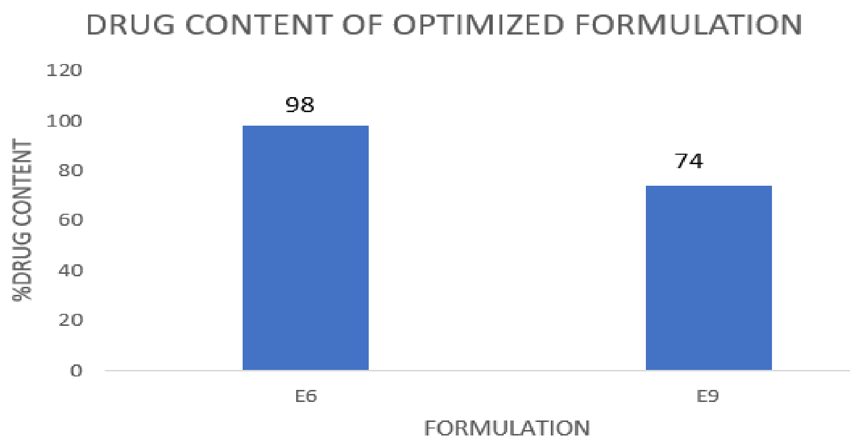


Figure 4.8.3: Drug content of optimized ethosomal formulations

The drug content values of the two best formulations were compared. Out of the two formulations the E6 formulation containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest drug content of 98%.

Entrapment Efficiency:

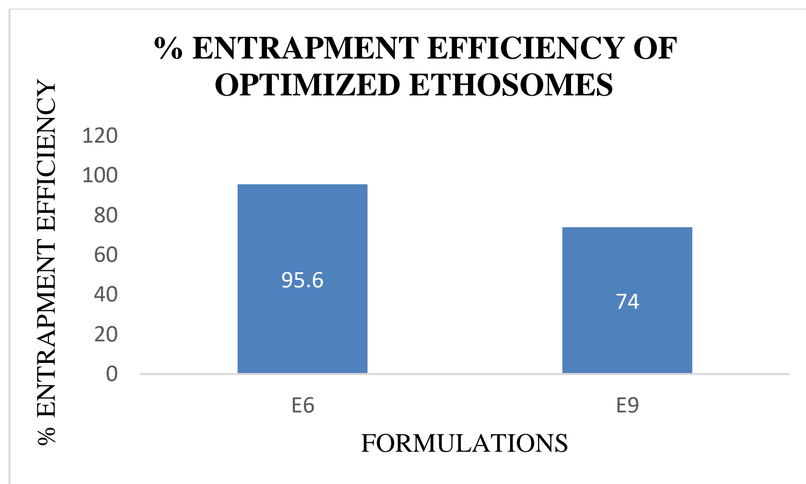


Figure 4.8.4: Entrapment efficiency of optimized ethosomal formulations

The entrapment efficiency of the two best formulations were compared. Out of the two formulations the E6 formulation containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest entrapment efficiency of 95.6%.

In Vitro Diffusion Studies:

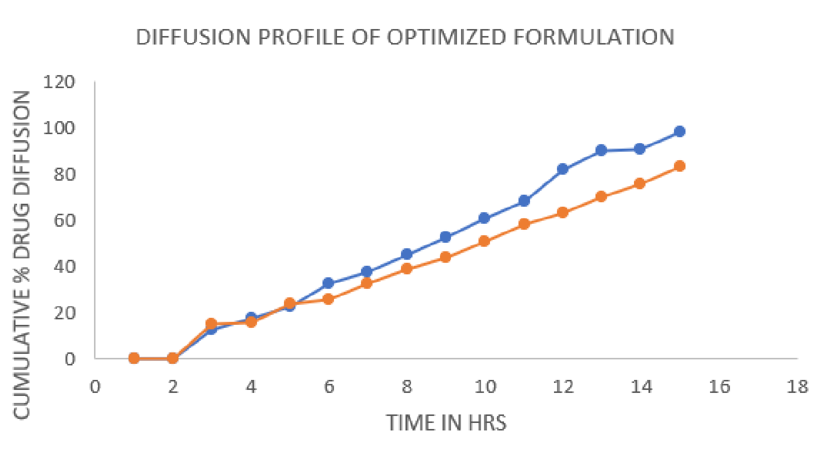


Figure 4.8.5: % drug release profile of optimized ethosomal formulations

The invitro drug release studies of the two best formulations were compared. Out of the two formulations the E6 formulation containing 1:6 ratio of drug to lipid was considered the better formulation because of its highest drug release of 98%.

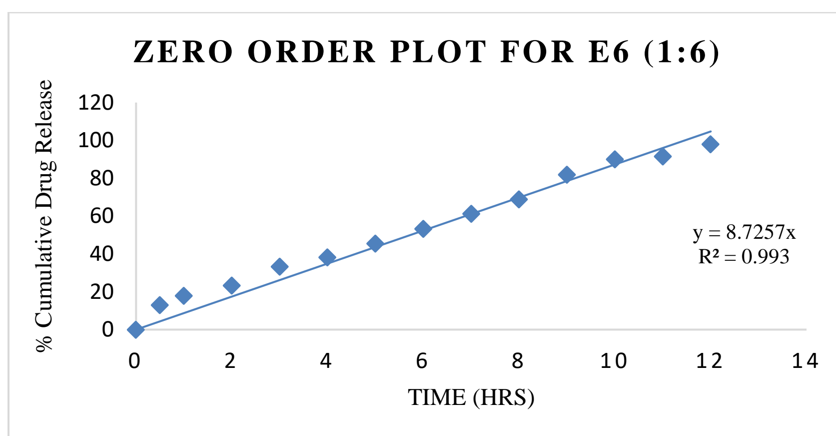
Table 4.9: Invitro drug release data of E6 best formulation of withania somnifera loaded ethosomes by cold method.

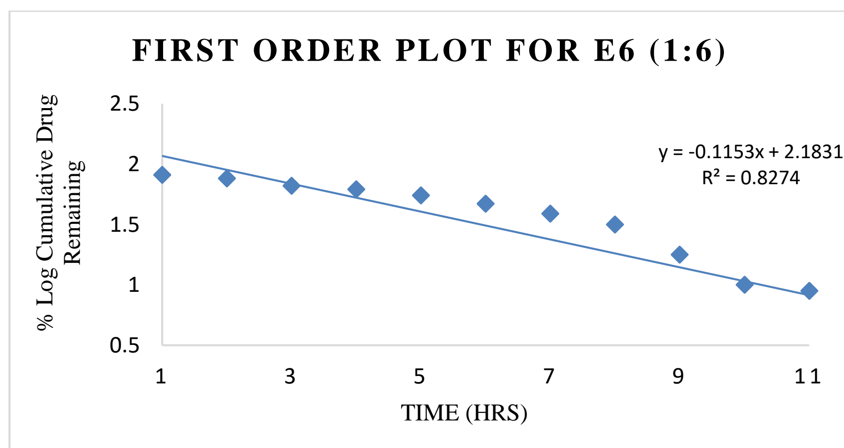
Time (hr.)	Cumulative drug release (%)	Drug remaining (%)	Log cumulative drug remaining (%)	$\sqrt{T}$	Log T	Log cumulative drug release (%)
0.5	13	87	1.93	0.70	-0.30	1.11
1	18	82	1.91	1	0	1.25
2	23.4	77	1.88	1.41	0.30	1.36
3	33.3	67	1.82	1.73	0.47	1.51
4	38.2	62	1.79	2	0.60	1.57
5	45.6	55	1.74	2.23	0.69	1.65
6	53.3	47	1.67	2.44	0.77	1.72
7	61.3	39	1.59	2.64	0.84	1.78
8	68.8	32	1.50	2.82	0.90	1.83
9	82	18	1.25	3	0.95	1.91
10	90.1	10	1	3.16	1	1.95
11	91.6	9	0.95	3.31	1.04	1.95

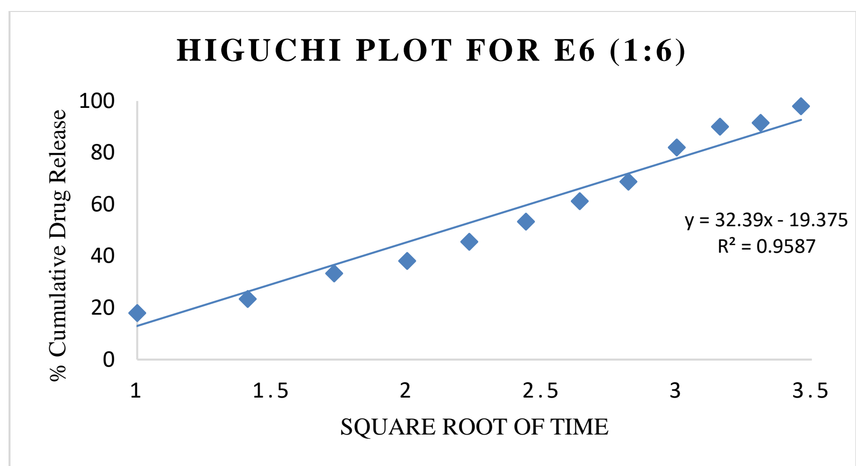
12	98	2	0.30	3.46	1.07	1.99

Drug Release Kinetic Plots for E6 Ethosomal Formulation by Cold Method:

Several plots (zero order plot, first order plot, higuchi plot and peppas plot) were drawn for the optimized ethosomal formulation by cold method in order to know the release kinetics and drug release mechanism.







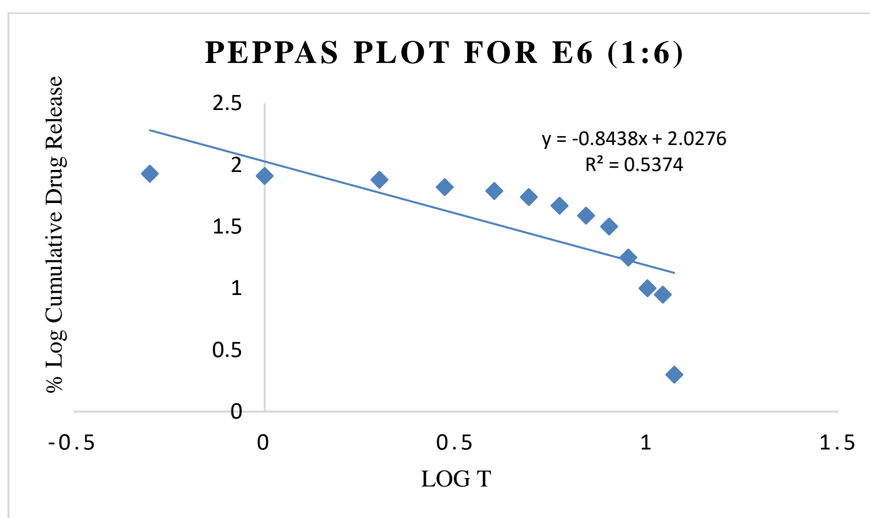


Figure 4.9.1: Comparison of optimized Ethosomal formulation E6 by cold method with various kinetic models.

Table 4.9.2: Kinetic data of E6 formulation of ethosomes by cold method.

Formulation	Zero order plot R2	First order plot R2	Higuchi plot R2	Peppas plot (n)
E6	**0.993**	0.827	0.958	**0.843**

From the result it was concluded that the drug release was following zero order kinetics and fitted into korsmeyer equation revealing non fickian diffusion mechanism.

DISCUSSION

Withania somnifera is a medical herb. It is safe and harmless. It is having both anti-inflammatory and anti-rheumatism properties. Eventhough Withania somnifera possess these properties but the basic problem is poor bioavailability. So, there is a need to develop Withania somnifera into novel vesicular drug delivery system i.e. ethosomal drug delivery system which results in particle size reduction and will enhances the penetration through skin and also increases bioavailability. The dried extract was obtained by extracting with ethanol, n hexane and ethyl acetate using soxhlet apparatus.

The dried extract was studied for the presences of major active constituents. By performing TLC it was observed that steriods is the major component of the extract. Rf values were observed for different bands and the value were found to be 0.88 for the standard withanolide and 0.99 for the root extract of withania somnifera which indicates the presence of withanolide as major compounds. The obtained extract was studied for anti-microbial activity, the ethanol and ethyl acetate extract of roots of withania somnifera was showing better Antimicrobial activity against Staphylococcus aureus with a zone of inhibiton30 mm, E. coli with a zone of inhibition of 26 mm and pseudomonas argenosa with 31 mm compared to streptomycin disc with a zone of 25 mm respectively.

So, to improve the bio-availability ethosomal formulation was selected by two methods i.e. hot method and cold method to prepare ethosomes of withania somnifera by using soya lecithin as lipid, ethanol, propylene glycol as solvents.

The objective of the present research work is to prepare and evaluate Withania somnifera loaded Ethosomal formulation by using both cold and hot methods.

Total twelve formulations (E1-E12) of Withania somnifera loaded ethosomes were prepared.

First six formulations (E1, E2, E3, E4, E5 and E6) were prepared by cold method by varying drug to lipid ratio (1:1, 1:2, 1:3, 1:4, 1:5 and 1:6) i.e., by increasing lipid concentration (10mg, 20mg, 30mg, 40mg, 50mg and 60mg). Among the six formulations (E1-E6) the E6 formulation containing 1:6 ratio of drug to lipid is giving the better result with good drug content, entrapment efficiency, zeta potential, mean particle diameter and invitro drug release.

Other six formulations (E7, E8, E9, E10, E11 and E12) were prepared by hot method by varying drug to lipid ratio (1:1, 1:2, 1:3, 1:4, 1:5 and 1:6). i.e., by increasing lipid concentration (10mg, 20mg, 30mg, 40mg, 50mg and 60mg). Among the six formulations (E7-E12) the E9 formulation containing 1:3 ratio of drug to lipid is giving the better result with good drug content, entrapment efficiency, zeta potential, mean particle diameter and invitro drug release.

Successful ethosomal formulations were prepared for Withania somnifera. On comparison of all prepared ethosomal formulation (E1-E12) of Withania somnifera the E6 formulation prepared by cold method containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest drug content of 98%, entrapment efficiency of 95.6%, particle size of 254.6 nm, zeta potential of -29.4mV and drug release of 98% in a time period of 12 hrs.

In this present study Withania somnifera loaded ethosomes were successfully prepared and evaluated.

CHAPTER-5

CONCLUSION

CONCLUSION

In the present study Withania somnifera loaded ethosomes were prepared and evaluated. Withania somnifera is shrub which exhibits Anti-inflammatory and anti-arthritis activitiy.

The withania somnifera extract was obtained by Soxhlet extraction method. The obtained withania somnifera root extract was screened for Various Phytochemical constituents. The phytochemical screening tests revealed the presence of Tannins, flavonoids, alkaloids, saponins, steroids, and phenols. Further confirmation was done by TLC. The Rf value was found to be 0.88 for the standard withanolide and 0.99 for the root extract of withania somnifera. The Rf value revealed the confirmation of steriods as the major active constituent.

The cold and hot methods were adopted for the preparation of ethosomes for the withania somnifera extract.

Total twelve formulations (E1-E12) of Withania somnifera loaded ethosomes were prepared. First six formulations (E1- E6) were prepared by cold method by varying drug to lipid ratio. Other six formulations (E7-E12) were prepared by hot method by varying drug to lipid ratio. All the twelve formulations were evaluated for drug content, entrapment efficiency, particle size and zeta potential and invitro diffusion studies.

Out of the twelve formulations, the E6 formulation prepared by cold method containing 1:6 ratio of drug to lipid was considered the best formulation because of its highest drug content of 98%, entrapment efficiency of 95.6%, particle size of 254.6 nm, zeta potential of -29.4mV and drug release of 98% in a time period of 12 hrs.

So, in this research work Withania somnifera loaded ethosomes were successfully prepared and evaluated. The aim is achieved.

The objectives were fulfilled in the current research work.

CHAPTER-6

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ANNEXURE-1

LIST OF CO-CURRICULAR ACTIVITIES

- **LIST OF PRESENTATIONS:**
  - Presented poster presentation on “DEVELOPMENT OF VESICULAR DRUD DELIVERY SYSTEM FOR WITHANIA SOMNIFERA” in two-day national seminar on “Pharmaceutical Education and Research Challenges for Emerging Prospects & Trends” PERCEPT-2020 organized by Department of pharmacy, university college of technology, Osmania University on 13th and 14th march, 2020.
  - Presented poster presentation on “Formulation of Herbal ACNE CREAM FOR THE TREATMENT OF ACNE VULGARIS” during 12th indo-malaysian conference on “Innovations and updates in pharmaceutical sciences” held by RBVRR womens college of pharmacy on 22 th November 2019.
- **LIST OF PARTICIPATIONS**:
  - Participated in “2 nd indo-malaysian conference” on “Recent trends and challenges in pharmaceutical and clinical research” at RBVRR Women’s college of pharmacy on 6th October 2018.
  - Participated in “National level e-poster competition” on “Virtual Challenges in Teaching-Learning Process” held at RBVRR Women’s college of pharmacy during 10-25th may 2020.
  - Participated in “National level e-poster competition” on “COVID-19 Pandemic” organized by Dr. D. Y. Patil institute of pharmaceutical sciences and research.
  - Participated in “Connect Chancellor”, the state level competition held during COVID19 lockdown period in May 2020.
  - Participated in “IIC Online Sessions” conducted by “Institutions innovation council of MHRD’s innovation cell” from 28th April to 22nd may 2020 during COVID-19 nationwide lockdown.
  - Participated in “Online Quiz” entitled “Assess your pharmacotherapy proficiency” held by Guru nanak institutions on 16th September 2020.

- Participated in “International Quiz” on “Applications of Artificial Intelligence” organized by Parul University on 16 September 2020.
- Participated in “Pharma Quiz” on “Transforming global health” organized by RBVRR Women’s college of pharmacy on the occasion of World Pharmacist Day, 25th September 2020.
- Participated in “Online Quiz” on “Computer aided design and manufacturing” organized by TPEVR Government polytechnic college on 15th August 2020.
- Participated in “COVID-19 Pandemic general awareness quiz” organized by D.B.F Dayanand college on 12th August 2020.
- Participated in “Quiz” on “Clinical Pharmacology” held by Malla reddy college of pharmacy on 22nd may 2020.
- Participated in “Online Quiz” on “Novel drug delivery systems” organized by Sultan-ul-uloom college of pharmacy on 27th may 2020.

- **LIST OF PARTICIPATED WEBINARS**:
    - Participated in the webinar on “Artificial intelligence in drug discovery and healthcare system” held at RBVRR Women’s college of pharmacy during 4th -8 th August 2020.
    - Participated in one week online international skill development program on “Innovations in natural product driven drug discovery and analytical chemistry” held by shri Vishnu college of pharmacy from 06th to 11t July 2020.
    - Participated in international pharma webinar on “Skills and career opportunities for pharmacists-A global perspective” organized by MNR college of pharmacy on 30th may 2020.
    - Participated in mybo webinar-3 on “Optimization in pharmaceutical technology: concepts, applications and demonstration” organized by MYBO Group on 31st may 2020.
    - Participated in two-day webinar on “Design of experiment” organized by Vignan pharmacy college on 30th may 2020.
    - Participated in online webinar on “ICH Q10-A comprehensive model for pharmaceutical quality system” organized by Vignan pharmacy college on 31st may 2020.

- Participated in International virtual seminar on “Pragmatic approaches to create sustainable lifestyle management” held by Government first grade college on 5th & 6 th October 2020.
- Participated in international webinar on “Post covid global education: challenges and opportunities” organized by Cinnamara college on 17th August 2020.

ANNEXURE-2

ARTICLES