ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

FORMULATION AND EVALUATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS) OF *COMMIPHORA WIGHTTI* EXTRACT

Mahendra Kumar Singh*, Mahendra Pratap Singh, Alok Pal Jain, Kavita R. Lokesh

Department of Pharmacy, SRK University, NH-12 Hoshangabad Road, Misrod, Bhopal, Madhya Pradesh, India

**Corresponding author: shailgpharma@gmail.com*

ABSTRACT

Commiphora mukul widely known as Guggul herb is highly used by the rural and tribal people for curing various disorders. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. In the present study an attempt was made to formulate and develop self emulsifying drug delivery system (SEDDS) of methanolic extract of *Commiphora wightti* (guggul) and to enhance its solubility, dissolution and its bioavailability. The solubility of guggul extract was determined in various oil, surfactant and co-surfactants. Pseudoternary Phase diagram were used for optimization of microemulsion formulation. The prepared formulations of SEDDS were evaluated based on their *in-vitro* drug release profile, globule size, zeta potential and self emulsifying assessment. *In vitro* release studies of optimized formulation F3 revealed that release profiles of SEDDS of guggul extract was best expressed by Higuchi equation, as the plots showed highest linearity (coefficient of determination, R2=0.993). It was observed that the optimized SEDDS formulation F3 showed 98.14 % release at 60 min.

Keywords: *Commiphora mukul, SEDDS,* Microemulsion, Pseudoternary Phase diagram, Extract.

1. INTRODUCTION

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments. Scientists reported that plants contain a wide variety of active principles [1-3]. Phytochemicals are chemical compounds formed during the plants' normal metabolic processes and often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [4] . The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research [5]. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals [6]. There are several standard methods used for the phytochemical screening of medicinal plants. There are variety of phytoconstituents like alkaloids, steroids, saponins [7], phenolics [8], flavonoids [9], saponins and cardiac glycosides [10] and tannins [11]. *C. wightii* (Arn.) Bhandari is a well known herbal plant of Burseraceae family is commonly known as "Indian bedellium", "Mukul myrrh tree", "Gugal", "Gugulu or "Guggul" in India. It is widely distributed in tropical regions of Africa, Madagascar and Asia. In India it is found in Rajasthan, Gujarat and Maharashtra [12]. It is used in the Allopathic, Ayurvedic and Unani systems of medicines due to its anti-inflammatory, anti-rheumatic, hypocholesteremic and anti-fertility activities [13]. C. *wightti* yields guggul, an important oleo-gumresin which is complex mixture of resin (61 %), gum (29.3 %) and other chemicals (6.1 %) and used as incense, fixative in perfumery and in medicine [14]. *C. wightii* are known to contain chemical constituents belonging to different chemical groups, namely, alkaloids, glycosides, steroids, terpenoids, flavonoids, coumarins, tannins, and anthraquinones. The active constituent of gugulipid is guggulsterone, which is present in a concentration of 4.0-6.0%. The guggulsterone is present in guggulipid in the form of stereoisomers E-guggulsterone and Z-guggulsterone. Gugulipid is a potent hypolipidemic agent. Apart from its hypolipidemic activity, a large number of therapeutic activities like antimicrobial, anthelmintic, anti-inflammatory, antiarthritic and antioxidant have been reported [14,15].

2. MATERIAL AND METHODS

2.1. Collection and preparation of plant material

The stem barks of *Commiphora wightii* were collected from Bhopal. The plant was washed, chopped in to small pieces and dried under shade and powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

About 200 gm of coarsely powdered plant material was successively extracted by Soxhlet extraction method using solvents with increasing polarity *viz*. petroleum ether and methanol. Each time before extracting with next solvent, the powdered material was dried in hot air oven (mentioned temp range). Each extract was then concentrated by distilling off the solvent by evaporation to water bath. All the extracts thus obtained were stored in air-tight bottles at 4°C for further experiments [16].

2.2. Solubility Profile

Solubility of Guggul extract was checked visually in distilled water, methanol, ethanol, DMSO, chloroform, acetone and phosphate buffer (pH 5.4). Accurately weighed 1 gm of drug was transferred in a clean and dry test tube followed by addition of the solvents individually and shaken vigorously and the solubility of drug was checked visually.

2.2.1. Determination of solubility in DMSO and phosphate buffer pH 5.4 mixture for determination of ratio

Accurately weighed 20 mg of Guggul extract was added individually to ten clean and dried volumetric flasks each of 10 ml capacity each. DMSO and phosphate buffer, pH 5.4 solvent system was added in the ratio 1:9, 2:8, 3:7, 4:6,5:5, 6:3, 7:3, 8:2, 9:1 and the samples were shaken for 1 hour on linear motion shaker (Model no. REMI RQ 123, Spectra Whirlmatic Lab, India). The solutions were checked visually for their clarity.

2.3. Analytical Methodology

The ultraviolet absorption spectrum of a solution of Guggul extract in DMSO and (8:2) mixture of DMSO and Phosphate buffer pH 5.4 was obtained using UV/VIS spectrophotometer over a wavelength range of 200 to 400 nm. Maximum absorption (λmax) values were determined and further used for plotting calibration curve.

2.3.1. Characterization of Oil, Surfactant and Co-Surfactant for Microemulsion [17,18]

2.3.1.1. Solubility Determination in various Oil, Surfactant and Co-surfactant

Preformulation solubility analysis was done to select the vehicle in which drug is more soluble and suitable for formulation of SEDDS. The solubility of drug in various oils, surfactants and co surfactants was measured and the solvents for the study were selected based on the good solublising capacity for drug. In present study the solubility of drug was investigated in different oils like soyabean, olive oil, Capryol 90 etc, surfactants and co-surfactants like labrasol, Cremophor EL and PEG, Propylene glycol etc. An excess amount of drug was added into each vehicle followed by vortex mixing for 30sec (Remi mixer, Mumbai). Mixtures were shaken for 48 h at 30°C, followed by equilibrium for 24 hr. The equilibrated samples were then centrifuged at 1000 rpm for 10 min to remove the insoluble drug and clear supernatant liquid was decanted. An aliquot of the supernatant was diluted with DMSO and solubility of drug was estimated by UV spectroscopy at 328 nm.

2.3.1.2. Screening of oils and surfactant

The oils and surfactants were selected on the basis of their tendency for instant emulsification and solubility in extract. The oils selected for this investigation were Capryol 90, Olive oil and Soyabean oil. The surfactants selected were Cremophor RH-40, Labrasol and Cremophor EL. The oils and surfactant were mixed in a ratio of 1:1. Briefly, 150 mg of the surfactants were added to 150 mg of the oily phase. Each mixture (100 mg) was then diluted with distilled water to 100 ml in a stoppered conical flask. Ease of emulsification was judged by the number offlask inversions required to yield homogenous emulsion. Emulsions were allowed to stand for 2hr and their % transmittance was evaluated at 638 nm by UV-Visible spectrophotometer using distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation.

2.3.1.3. Preliminary screening of co-surfactants

The selected oily phase and surfactant were used for further screening of the different co-surfactants (Propylene glycol, PEG 400 and Tanscutol) for their emulsification ability. Mixtures of 200 mg of co-surfactant, 400 mg selected surfactant, and 600 mg screened oil were prepared and evaluated in a similar fashion as described in preliminary screening of surfactants.

2.3.1.4. Pseudo Ternary Phase Diagram

On the basis of the solubility data presented in Table, Capryol was selected as oil, labrasol as surfactant and PEG-400 as co-surfactant. In pseudo-three-component phase diagram, one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and co-surfactant at fixed weight ratios (Smix ratio). Pseudoternary phase diagrams of oil, surfactant/cosurfactant and water were developed using the water titration method.

Surfactant and co-surfactant (Smix) in each group were mixed in different weight ratio of (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1). For each phase diagram oil and specific Smix ratio were mixed thoroughly in different weight ratio from in different glass vials. Different combination of oils and Smix were prepared for the study to delineate the boundaries of phase precisely formed in the phase diagrams. The mixtures of oil and Smix at certain weight ratios were diluted with water in a drop wise with constant stirring on 6 Station magnetic stirrers until homogeneous dispersion or solution was obtained. The end point of the titration was the point in which the solution becomes cloudy or turbid. The quantity of aqueous phase required to achieve turbidity point was noted.

2.4. Formulation of Self Emulsifying Drug Delivery System [19, 20]

Self Emulsifying Drug Delivery system of Gum guggul extract was formulated by mixing oil, surfactant and co-surfactant with varying component ratio. In all the formulations, amount of guggul extract was kept constant and varying ratio of oil and surfactant and co surfactant mixture were added. The required amount of Gum Guggul extract was dissolved in selected oil at room temperature by permanent agitation and then mixture of surfactant and co-surfactant were added with gentle stirring and sonication. Then an appropriate amount of water was added to the mixture drop wise with constant stirring. Micro emulsion of guggul extracts was obtained spontaneously on stirring the mixture at ambient temperature. The various formulation ratios (F1 to F8) are given in Table 1. The process of self-emulsification was visually monitored for the rate of emulsification and for the appearance of the produced emulsions. The visual properties registered against the increment of the applied surfactant component in Ternary triangular diagrams. Plotting points of preferential combinations were selected according to calculation.

Table 1: Compositions of Guggul extract (SNEDDS) (1–8). The components were Capryol as oily phase, Labrasol or Cremophore as surfactant, and Propylene glycol as co surfactant. The concentration of Guggul extract was 10mg/mL in all samples

Compositions	Formulation code	Capryol	Propylene Glycol	Cremophore EL	Labrasol
Composition 1	F1	33 %	33 %	33 %	-
Composition 2	F2	25 %	50 %	25 %	-
Composition 3	F3	20 %	60 %	20 %	-
Composition 4	F4	25 %	25 %	50 %	-
Composition 5	F5	33 %	33 %	-	33 %
Composition 6	F6	25 %	50 %	-	25 %
Composition 7	F7	15 %	60 %	-	15 %
Composition 8	F8	25 %	25 %	-	50

2.5. Evaluation of Formulation Self Emulsifying Drug Delivery System [19-25]

2.5.1. Drug excipient compatibility studies

A proper design and formulation of the dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in the fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. If the excipients(s) are new and if no previous literature regarding the use of those particular excipients with an active ingredient is available, then compatibility studies are of paramount importance. Infrared (IR) is related to covalent bonds, the spectra provided detailed information about molecular structure. Hence, before producing the actual formulation, compatibility of NVP with different polymers and other excipients were tested using the Fourier transform infrared (FT-IR) spectroscopy technique.

Fourier transforms infrared spectroscopy is a useful analytical technique utilized to check the chemical

interaction between drug and other excipients used in the formulations. Drug and the intended excipients interaction were studied by FT-IR. The intended samples were powdered and intimately mixed with dry powdered potassium bromide. The powdered mixture was taken in a diffuse reflectance sampler, and the spectrum was recorded by scanning in the wavelength region of 4000-400 cm^{-1} in FT-IR spectrophotometer.

2.6. Drug content

Self-emulsifying drug delivery systems formulation equivalent to 100 mg of guggul extract was taken and dissolved in small quantity of methanol. Volume was made up to 100 ml with DMSO solution (1 mg/ml). From the above stock solution, 0.2 ml (200µg/ml) was withdrawn and diluted up to 10 ml with methanol (20µg/ml). Samples were prepared in triplicate and absorbance measured at 328 nm using UV-visible spectrophotometer. DMSO was used as a reference solution.

2.7. Self emulsification assessment

SMEDDS should form stable microemulsion instantaneously in GI fluids upon administration. Efficiency of selected combination of surfactant and co-surfactant in self microemulsification was assessed by dispersing the SMEDDS in 250 mL of water with magnetic stirring at 100 rpm to create gentle turbulence that mimic in vivo condition and assessed visually.

2.8. Determination of droplet size and zeta potential

The charge of the droplets was determined by zeta potential measurement. Zeta potential helps in predicting the flocculation effect and stability in emulsion systems. Colloid will aggregate due to attractive forces if the zeta potential falls below a certain level. Droplet size and the zeta potential of the formed emulsion were determined using Zetasizer (Nano ZS 90, Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90°angle.

2.9. In vitro *dissolution technique*

The quantitative *in vitro* dissolution studies are carried out to assess drug release from oil phase into aqueous phase by USP type II dissolution apparatus use of 900 ml of pH 6.8 phosphate buffer solution at 75 rpm and maintain the temperature at 37°C±0.5°C. Aliquots of 5 ml samples were withdrawn at regular intervals of time (5, 10, 15, 30, 60 min) and volume withdrawn was replaced immediately with fresh medium. Samples taken were then analyzed by use of UV spectrophotometer at 328 nm.

2.10. Evaluation of anti-hyperlipidemic effect of Optimized formulation [26-27]

2.10.1. Diet-induced hyperlipidemic model

The animals were selected, weighed then marked for individual identification. Rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days. The rats were then given plant extracts suspended in 2% acacia at the dose of 200mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days.

During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.

2.10.2. Triton-induced hyperlipidemic model

Animals kept for fasting for 18 h, were injected a saline solution of Triton (Triton x-100) at the dose of 100mg/kg b.w. intra-peritoneally. The plant extracts, at the dose of 200mg/kg b.w., was administered orally through gastric intubation. The first dose was given immediately after triton injection and second dose 20 h later and continued the extraction process for 7 days. After 7 days dose the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.

2.10.3. Collection of blood samples

Blood sample (0.2 ml), was collected serially through retro orbital puncture at 15 & 24 hrs. After leaving the blood to clot for 30 min at room temperature, serum was separated by centrifugation. Then serum was examined for the assessment of biochemical parameters such as triglycerides, TC, LDL, VLDL and HDL, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, creatinine, albumin, and blood urea nitrogen.

2.10.4. Accelerated Stability Studies

The optimized formulations SEDDS were filled in the glass vial, sealed with rubber cap and crimped for storing in the stability chamber. Samples were subjected to a stability testing for six months as per ICH norms at a temperature and RH of 40°C±2C/75% RH±5% RH respectively. The selected formulations were analyzed for the change in droplet size, zeta potential, self-emulsification capacity and drug content [28].

3. RESULTS AND DISCUSSION

3.1. Solubility Profile

Solubility of powdered extract of Gum guggul in various solvent are presented is in Table 2. It can be revealed from table that Gum guggul is soluble in DMSO, thus DMSO was selected for further studies.

Table 2: Solubility Profile of Gum guggul extract

Solvent	Solubility
Distilled Water	Insoluble
Methanol	Sparingly Soluble
Ethanol	Sparingly Soluble
Acetone	Sparingly Soluble
Chloroform	Sparingly Soluble
Phosphate Buffer (5.4 pH)	Sparingly Soluble
DMSO	Soluble

3.2. Analytical Methodology UV-Visible Spectroscopy

The Lamda max (λ_{max}) of Gum guggul extract in DMSO was found to be 328 nm.

3.3. Characterization of Oil, Surfactant and Co-Surfactant

3.3.1. Selection of Excipients

In this study, we selected Cremophor EL and Labrasol as a surfactant. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a co surfactant is necessary. The presence of co surfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsions over a wide range of composition. Thus, the co surfactant selected for the study was Propylene glycol, which has an HLB value of 5-6. The surfactants and co-surfactants were selected on the basis of their emulsification efficiency and ability to solubilise guggul extract (Table 3).

Table 3: Solubility Determination of Gum guggul in Various Oil, Surfactant and Co-surfactant

Solvent		Solubility (µg/ml) in gum guggul
Oil	Olive Oil	24.1
	Capryol 90	65.4
	Soyabean Oil	32.5
Surfactant	Cremophor EL	71.23
	Labrasol	74.4
	CremophorRH-40	70.4
Co-surfactant	Propylene Glycol	56.2
	PEG 400	38.9
	Transcutol P	40.3

3.3.2. Pseudo-Ternary Phase Diagram

Phase diagrams of the systems containing Capryol as an oil phase, Labrasol & Cremophor EL as a surfactant and Propylene glycol a co-surfactant were constructed at the surfactant/co-surfactant (Smax) ratio of 1:3, 1:2, 1:1 and 2:1 (w/w) to determine the existence of micro-emulsion region. The phase study revealed that the obtained microemulsion region of composition 8 Smax ratios was low while composition 3 Smax ratio was maximum microemulsion region when compared with all other ternary plots. Study of ternary phase diagrams revealed that increase in the microemulsion regions gradually as concentration of co-surfactant increases; it indicates that the co-surfactant has some effect on the capability of forming micro emulsion. The composition 3 ratio of Smax showed maximum microemulsion region when compared to all other ternary plots, which points that an increase in the concentration of surfactant gives the highest microemulsion regions among all other ternary plots. It indicates that the concentration of surfactant has a major effect on the microemulsion region forming capability of SEDDS (fig.1).

3.3.3. Drug content

Assay of prepared guggul extract SEDDS was carried out by UV-visible spectrophotometer. A linear calibration curve was obtained at 328 nm in the range of (2-10µg/ml) with a correlation coefficient (R^2) of 0.998. The % drug content of all SEDDS formulations was found to be within the acceptable limits of drug content test. The Assay results are shown in table no.4.

3.3.4. Standard calibration curve

Standard calibraction curve of *Commiphora mukul* extract was determined by plotting absorbace vs concentraction at 328 nm and it follow the beer's law. The results are shown in Table 5 and Fig.2.

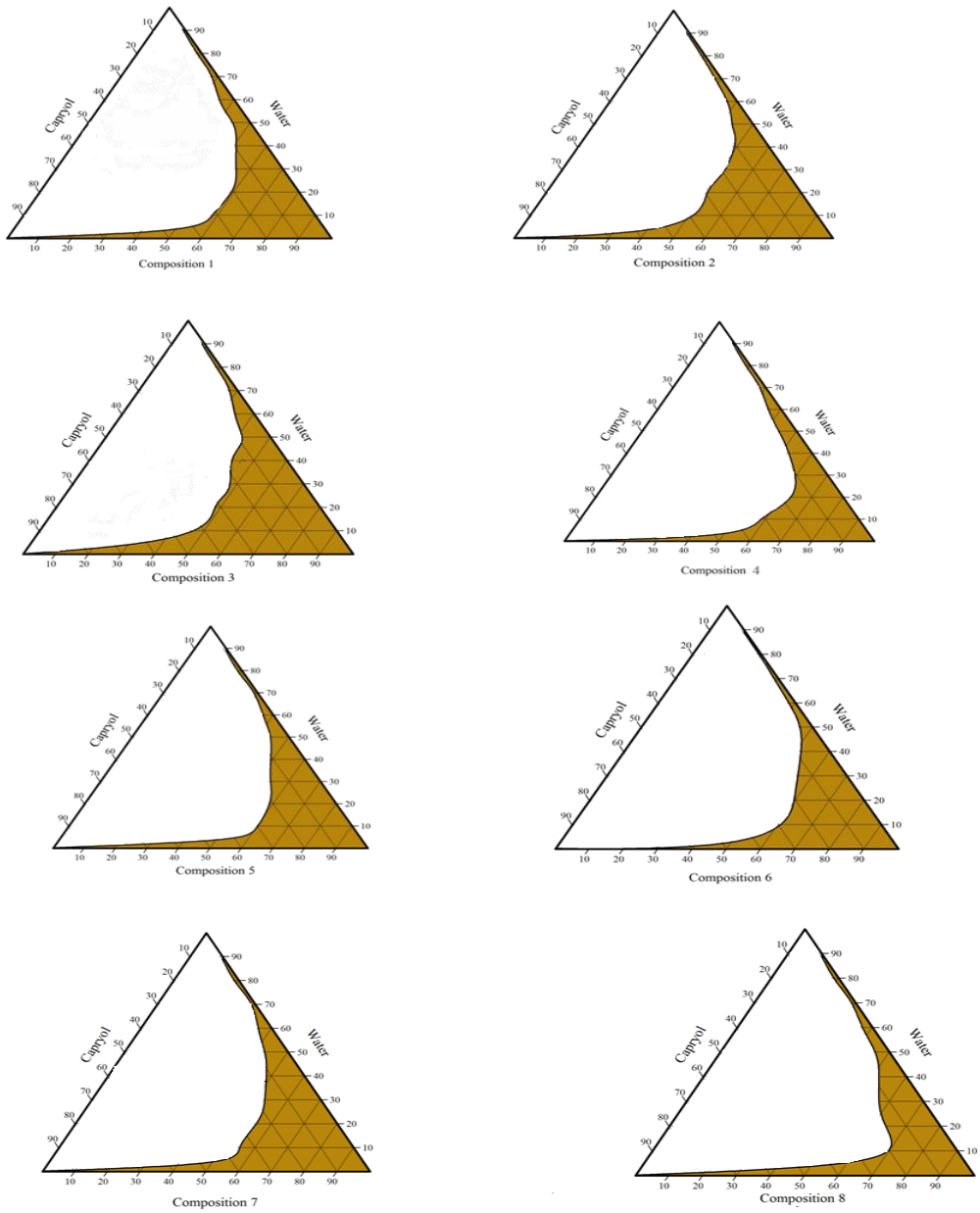

Fig. 1: Phase diagrams of the different composition formulation

Table 4: Drug Content of SEDD formulation with guggul extract

Formulation	Percentage of Drug contents (X±SD)
F1	97.3±1.7
F2	98.6±0.5
F3	99.3±0.7
F4	97.4±96
F5	98.6±0.9
F6	98.6±0.8
F7	98.9±1.3
F8	96.5±1.8

SD = Standard deviation

3.3.5. Determination of self-emulsification time

Emulsification time is an important index for the assessment of the efficiency of emulsion formation. SEDDS should disperse completely and rapidly when subjected to aqueous dilution under mild agitation. Formulation should disperse quickly when subjected to aqueous dilution under gentle agitation of GIT due to peristaltic activity. The emulsification time of all formulations was reported in Table. The lowest emulsification time 58 seconds was found in F3 formulation and highest emulsification time 170 seconds was found in F8 formulation. After observation, it was

found that the F3 formulation forms microemulsion in a short time relatively among all other formulations which indicate that the F3 was best of all prepared formulations.

Table 5: Standard calibration curve of *Commiphora mukul* at 328 nm

Concentration (µg/ml)	Absorbance
Blank	0.000
2	0.097
4	0.168
6	0.239
8	0.319
10	0.396

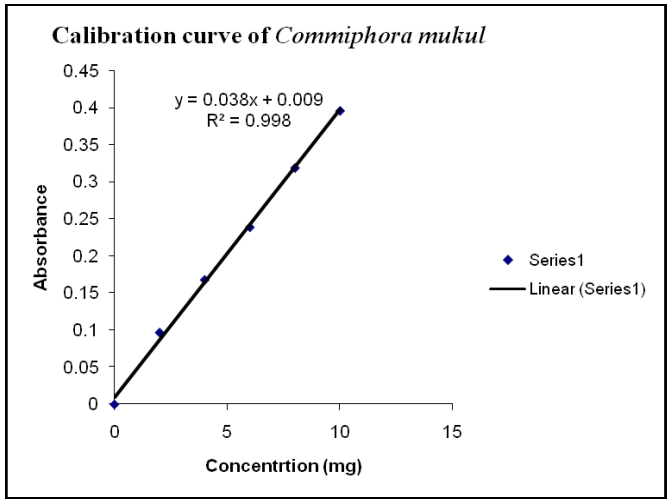

Fig. 2: Standard calibration curve of *Commiphora mukul* at 328 nm

Table 6: Self Emulsification time of SEDDS formulation

Formulation	Self Emulsification Time
F1	96 ±1.8
F2	67 ±0.4
F3	58 ±0.6
F4	120 ±2.8
F5	98 ±0.7
F6	97 ± 5.6
F7	62 ±1.3
F8	170±2.4

3.3.6. Droplet size analysis

The droplet size of the emulsion determines the rate and extent of drug release as well as absorption. The smaller is the droplet size, the larger is the interfacial surface area provided for drug absorption. There is a relationship between the droplet size of the emulsion and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to smaller mean droplet size. This may be due to the stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil-water interface. On the other hand, in some cases, the mean droplet size may increase with increase in surfactant concentrations. This phenomenon could be attributed to the interfacial disruption elicited by increased water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase.

The droplet size of the formulations is given in Table. It was found that as the surfactant and cosurfactant concentration increased, the droplet size was decreased. This may be due to more surfactant being available to stabilize the oil-water interface. However, a higher level of surfactant in Smax mixture resulted in the loss of flowability. Formulation F3 shows minimum droplet size i.e. 190.8 nm.

3.3.7. Determination of Zeta potential

The emulsion stability is directly related to the magnitude of the surface charge. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. For small molecules and particles, a high zeta potential will indicate stability means the system will resist aggregation. With low zeta potential the attractive forces exceeds repulsive forces which results in flocculation and breaking of the system. Several studies have reported that the zeta potential played an important role in the interactions with mucus of the gastrointestinal tract. According to the studies, the positive charged droplets could have better interaction with the mucus of the gastrointestinal tract, since the intestinal cell interior carry negative charges with the presence of mucosal fluid. The zeta potential value of the formulations is given in Table. The formulations have different positive zeta potential values. The F3 formulation shows maximum zeta potential value *i.e.* 16.2 mV.

Table 7: Droplet size and zeta potential of formulation

Formulation Code	Droplet size (nm)	Zeta potential (mV)
F3	190.8 nm	16.2 mV
F7	215.3 nm	13.3 mV

3.4. In vitro *dissolution study*

In vitro dissolution study was performed to compare the pure extract release from the developed guggul extract SEDDS formulations. The quantitative *in vitro* dissolution studies are carried out to assess drug-release from the oil phase into the aqueous phase by USP type II dissolution apparatus.

The results of *In vitro* dissolution studies were listed in table 8 and Fig. 3. After observing the results, it was found that, nearly 98.14% of drug was released from guggul SEDDS F3 formulation within 60 min compared to the other formulations, that is, F1, F2, F4, F5, F6 ,F7 and F8 which released 68.45% , 85.21%, 58.81%, 63.45%, 79.81,89.56 % and 52.45% of the drug respectively. Thus, the drug release from the guggul SEDDS F3 formulation was found to be significantly higher as compared to that of the remaining SEDDS formulations and pure extract. It could be suggested that the SEDDS F3 formulation resulted in a spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase. Thus, this greater availability of dissolved guggul extract from the SEDDS F3 formulation could lead to higher absorption and higher oral bioavailability.

The release data obtained in this study were extrapolated by the zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell equations to know the mechanism of drug release from the formulations. The in vitro drug release profiles of optimized formulation F3 was best expressed by Higuchi equation as the plots showed highest linearity (Coefficient of determination, R2 = 0.993). The formulations showed good linearity when plotted according to Higuchi equation. It can be inferred that the release was dependent on both motility and polymer relaxation.

Table 8: *In-vitro* drug release profile of SEDDS formulation

Time	SEDDS Formulation							
	F1	F2	F3	F4	F5	F6	F7	F8
5	22.63	27.85	32.85	18.45	19.39	22.72	29.4	15.45
10	39.32	40.88	47.88	31.98	34.82	36.64	39.67	25.61
15	44.23	50.89	54.55	40.43	41.32	49.76	50.16	33.56
30	53.83	63.68	72.68	48.54	49.37	60.48	66.35	43.54
45	62.25	74.45	88.45	53.57	59.61	69.95	78.68	48.89
60	68.45	85.21	98.14	58.81	63.45	79.81	89.56	52.45

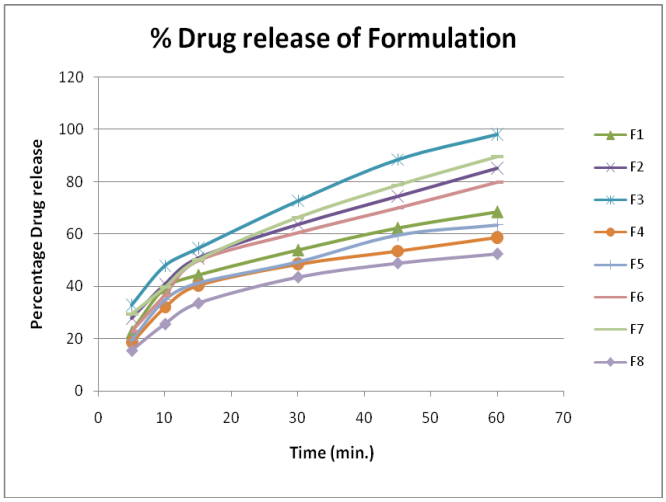

Fig. 3: *In-vitro* drug release profile of SEDDS formulation

3.5. Transmission electron microscope (TEM)

Droplets produced upon dilution of SMEDDS were visualized under high magnification and the same was illustrated. Spherical droplets were observed and droplet size was corroborative to dynamic light scattering measurements.

3.6. Pharmacological Screening

As reported earlier, Injection of High fat diet and Triton X-100 (100 mg/kg) has successfully induced hyperlipidemia in rats by increasing the serum TC, TG and LDL-C levels. The effect of methanolic extract of *Commiphora Wightii* on serum lipid profile levels was showed in Table 9 and 10. Treatment with formulation F-3 at the doses of 250 mg/kg significantly reduced the serum TC, TG and LDLC levels and increased the serum HDL-C levels when compared to the hyperlipidemic control group. The change in lipid levels in groups of II, III and IV were comparable with group of fenofibrate treated rats. Among two fractions, reduced the elevated lipid levels more significantly than the others.

Thus to conclude, the study showed that administration of *Commiphora Wightii* at dose level 250mg/kg was

effective as hypolipidemic agent. The active ingredient present in plant may recover the disorders in lipid metabolism noted in hyperlipidemic state and further work would be necessary to evaluate the active constituents responsible for the activity and mechanisms of these effects.

The *Commiphora Wightii* showed protective action at a dose of 250mg/kg and demonstrated a significant decrease in the raised diet-induced levels of serum TC, LDL-C and triglycerides. At a dose of 250mg/kg, effects were comparable with that of the standard drug fenofibrate.

Model-I High Fat Diet Induced Hyperlipidemia

Table 9: Effect of SEDDS formulation of Methanolic extract of *Commiphora Wightii* on serum biochemical parameters in cafeteria fed diet rats

Groups	Cholesterol (mg %)	TG_s (mg %)	HDL (mg %)	LDL (mg %)	VLDL (mg %)
Normal Control (2% CMC)	133.98±0.50	75.05±2.33	53.79±0.93	65.18±0.75	15.01±0.51
High fat caféteria diet	146.81±0.88^{a***}	95.53±2.12^{a***}	21.43±0.76^{a***}	106.28±0.29 ^{a***}	19.10±0.73^{a**}
Cafeteria diet + Feno-fibrate(65mg /kg/p.o.)	117.29±0.74$^{a***, b***}$	24.98±2.27$^{a***, b***}$	30.98±0.79$^{a***, b***}$	82.3±0.11$^{a***, b***}$	4.99±0.61$^{a***, b***}$
Cafeteria diet + SEDDS, F3 (250 mg/kg/p.o.)	121.40±1.47$^{b***, c***}$	32.81±3.09$^{a*, b***, c***}$	40.57±1.02$^{a***, b***}$	86.47±0.33$^{a***, b***}$ ^{c***}	9.36±0.71$^{a**, b***, c***}$

*Data are expressed in mean ± SEM, n = 6 (no. of six animals) in each groups,*p<0.05, **p<0.01, ***p < 0.001 compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.*

a-significant difference in compared with vehicle treated groups, b-significance difference in compared with high fat diet groups, c-significance difference in compared with standard drug treated groups, d-significance difference in compared with test drug treated groups.

Model-II Triton Induced Hyperlipidemia

Table 10: Effect of SEDDS Formulation of Methanolic extract of *Commiphora Wightii* on serum biochemical parameters in Triton induced hyperlipidemia in rat.

Groups	Cholesterol (mg %)	TG_s (mg %)	HDL (mg %)	LDL (mg %)	VLDL (mg %)
Normal Control (2% CMC)	110.34±1.1	90.15±2.7	45.34±2.2	46.97±0.00	18.03±0.01
Triton Control	152.51+4.3 ^{a***}	153.24+4.0 ^{a***}	27.65+1.6 ^{a**}	94.21±0.06 ^{a***}	30.62±0.02 ^{a***}
Triton + Fenofibrate (65mg/kg/p.o.)	120.15±2.3 ^{b***}	90.44+7.5 ^{b***}	55.84 +1.1 ^{b***}	46.26±0.00 ^{a***,b***}	18.05±0.00 ^{b***}
Triton + SEDDS,F3 (250mg/kg/p.o.)	121.40±1.47 $^{b***, c***}$	104.81±3.09 $^{a*, b***, c***}$	57.58 ±1.02 $^{a***, b***}$	50.47±0.33 $^{a***, b***}$ ^{c***}	20.36±0.71 $^{a**, b***, c***}$

*Data are expressed in mean ± SEM, n = 6 (no. of six animals) in each groups,*p<0.05, **p<0.01, ***p < 0.001 compared with multiple group using One-way ANOVA followed by Tukey multiple comparison test.*

a-significant difference in compared with vehicle treated groups, b-significance difference in compared with high fat diet groups, c-significance difference in compared with standard drug treated groups, d-significance difference in compared with test drug treated groups.

Table 11: Stability studies of Formulation

Formulation Code	Droplet size (nm)	Zeta potential (mV)	Self emulsification time (min)	Drug contents
F3	190.8 nm	16.1 mV.	194.4 nm	98.23
F7	215.3 nm	13.3 mV	12.82mV	97.83

3.7. Stability studies

Samples from stability chamber were withdrawn at regular intervals and evaluated for self emulsification efficiency, droplet size and zeta potential measurements. Results were represented in Table. There was no significant change in the droplet size, zeta potential and self-emulsification capacity. Clear dispersion with closer droplet size with initial samples indicates the stability of SMEDDS.

4. CONCLUSION

The results indicate that effective SEDDS formulation of methanolic extracts *Commiphora Wightii* prepared and SEDDS formulation at the doses of 250 mg/kg significantly reduced the serum TC, TG and LDLC levels and increased the serum HDL-C levels and give promising results.

The effect of ethanolic extracts formulation SEDDS of *Commiphora Wightii* at the doses of 250 mg/kg significantly reduced the serum TC, TG and LDLC levels and increased the serum HDL-C levels when compared to the hyperlipidemic control group. The change in lipid levels in groups of II, III and IV were comparable with group of fenofibrate treated rats.

Thus to conclude, the study showed that administration of *Commiphora Wightii* at dose level 250mg/kg was effective as hypolipidemic agent. The active ingredient present in plant may recover the disorders in lipid metabolism noted in hyperlipidemic state and further work would be necessary to evaluate the active constituents responsible for the activity and mechanisms of these effects.

The *Commiphora Wightii* showed protective action at a dose of 250mg/kg and demonstrated a significant decrease in the raised diet-induced levels of serum TC, LDL-C and triglycerides. At a dose of 250mg/kg, effects were comparable with that of the standard drug fenofibrate.

5. REFERENCES

1. Adebajo AO, Adewumi CO, Essein EE. *International Symposium of Medicinal Plants.* (5th edn.), 1983; University of Ife, Nigeria: 152-158.
2. Agrawal H, Kaul N, Paradkar AR, Mahadik KR. *Journal of Pharmaceutical and Biomedical Analysis,* 2004; **36(1):**33-41.
3. Awe IS, Sodipo OA. *Nig. J. Biochem. Mol. Biol*. 2001; (Proceedings Supplement) **16:**201-204.
4. Banso A, Adeyemo SO. *Nig. J. Biotech*., 2007; **18(1-2):**27-32.
5. Boham BA, Kocipal-Abyazan R. *Pacific Science,* 1974; **48:**458-463.
6. Cowman MM. *Clinical Microbiology Revie*w, 1999; **12:**561-582.
7. Eshrat HM, Hussain A. *Indian Journal of Clinical Biochemistry,* 2002; **17(2).**
8. Fatope MO, Al-Burtomani SKS, Ochei JO, Abdulnour AO, Al- Kindy MZ, Takeda Y. Muscanone: a 3-O- (10', 80' 140'-trimethylhexadecanyl) naringenin from *Commi-phora wightii*. *Phytochemistry,* 2003; **62:** 1251-1255.
9. Harbone JB. *Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis* (3rd edn.). 1998, London, New York, Chapman and Hall Co.
10. Harbone SB, Baxer H. 1995. *Phytochemical dictionary- A handbook of bioactive compounds from plant*. London, Taylor and Francis.
11. Kokate CK. 1994. *Practical Pharmacognosy* (4th edn.). New Delhi, India. Vallabh Prakashan: 112-120.
12. Kokate CK. 2007. *Practical Pharmacognosy*. Pune, Nirali Prakashan.
13. Kumar S, Shanker V. 1982. Medicinal Plants of Indian Desert: *Commiphora wightii* (Arn.) Bhandari. *Journal of Arid Environments*, 5: 1-11.
14. Majorie MC. *Clin. Microbiol.,* 1999; **12(4):**564-582.
15. Ramawat KG, Mathur M, Dass S, Suthar S. *Bioactive molecules and medicinal plants*, 2008; Heidelberg, Springer: 101-121.
16. Trease GE, Evans WC. 1996. *A Textbook of Pharmacognosy*. (14th Eds.). London, Bailliere Tindall Ltd.
17. Gupta AK, Mishra DK. *Int J Pharm Life Sci.,* 2011; **2:**633-639.
18. Tejinder KM et al. *Journal of research and education in Indian medicine*, 2014; **XX(2):**75-87.
19. Gupta AK, Mishra DK, Mahajan SC. *Int. J. of Pharm. &Life Sci.,* 2011; **2(3):**633-639.
20. Chopade VV, Chaudhari PD. *Int J Res Dev Pharm Life Sci*., 2013; **2:**531-537.
21. Patel PA, Chaulang GM, Akolkotkar A, Mutha SS, Hardikar SR, Bhosale AV. *J Pharm Technol.,* 2008; **1:**54-68.
22. Sapraa K, Saprab A, Singha SK, Kakkarb S. *Indo Glob J Pharm Sci.,* 2012; **2:**313-32.
23. Harshal DM, Shaikh T, Baviskar D, Rajendra DW. *Int J Pharm Pharm Sci.,* 2011; **3 Suppl 4:**163-166.
24. Pandya D, Patel P, Patel S. *Discov Pharm*., 2013; **5:**6-12.
25. Kokare CR, Kumbha SA, Archna Patil. *Indian Journal of Pharmaceutical Education and Research*, 2013; **47(2).**
26. Rathod D, Dodiya H, Goswami S. *Inter-national Journal of Pharmacology,* 011; **7(6):**690-696.
27. Allenki Venkatesham et al, 2009; **2**(**5**):777-780.
28. ICH Harmonized Tripartite Guidelines, Stability Testing of New Drug Substances and Products. ICH Committee 2003; 8.