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Formulation, characterization and antimicrobial evaluation of *Thuja occidentalis* essential oil loaded lipid nanoparticles

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ABSTRACT

The objective of the present work was to prepare lipid nanoparticles encapsulated with Thuja oil with an objective to improve its stability and oral bioavailability. The results of preformulation study are identical to the specification reported for Thuja oil revealing yellow liquid with characteristic odor, a boiling point of 115°C and solubility in methanol and ethanol. All the prominent stretching and bending vibrations of Thuja oil were present in the physical mixture indicating a compatibility between the both the components. The preparation of the T-SLNs was achieved using probe-sonication method. Total eight formulations of T-SLNs were prepared by varying the concentration of the lipid, concentration of the surfactant and time of sonication. These formulations were assessed for particle size and encapsulation efficiency. The particle size of the formulations ranged between 111 ± 2.88 to 126 ± 168.56 nm. The encapsulation efficiency of the SLNs ranged between 71.23 ± 0.17 to 87.75 ± 0.30%.The particle size and zeta potential of the formulation T-SLN3 were 125 ± 2.64 nm and -21.7 ± 2.61 mV respectively. The stability of T-SLN3 was studied by storing at 4 ± 1 °C for 30 days. The particle size remained stable at the end of the study with drug entrapment of 81.3%. This suggests that the SLNs prepared are stable on storage. The T-SLN3 was evaluated for antibacterial action against *E. coli* and *S. aureus*. The SLN was able to exhibit significant antibacterial action against *S. aureus* whereas activity against *E. coli* was poor.

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Introduction

Essential oils have a long tradition in pharmaceutical sciences as natural products with pharmacological, cosmetic, agrochemical and nutritional applications[1]. These products have been used for centuries and are accepted in traditional or modern healthcare systems of medicine. Thuja oil (TO) is an essential oil obtained from the plant Thuja occidentalis and is mainly composed of Borneol, camphene, Fenchone, Limonene, α-Terpine , Terpinolene, Thujone (0.76-2.4 % of essential oil, 85% α-thujone, 15 % β-thujone), Thujylalcohol. Thujone is the highly toxic substance of Thuja species, showed the anticancer activity should be applied in clinical level so that further it may be used therapeutically in chemotherapies. After oral administration to male rabbits of a mixture of α- and β-thujone (ratio 9:2) at a dose level of about 650-800 mg/kg bw, two neutral urinary metabolites were identified as 3-- hydroxy-α-thujane and 3-- hydroxy-thujane indicating that the reduction was stereospecific in spite of the different configurations of the methyl group.[2]

α-thujone was rapidly metabolised by mouse liver microsomes forming 7-hydroxy-α-thujone as the major metabolite with five minor products (4-hydroxy-α-thujone, 4-hydroxy-- thujone, two other hydroxythujones and 7,8-dehydro-α-thujone).[2]

Several reports related to essential oils in lipid nanoparticles are available and all these reports reveal that essential oils have humongous therapeutic potential. The ability to avenge cancer or antioxidant action has been the most widely investigated potential of essential oils. It has been found to have effect on almost all the known chemotherapeutic targets of cancer. In spite of all the potential, essential oils are not marketed as drugs owing to their poor systemic bioavailability as well as stability issues.[3] Numerous reports have been made that describe that the formulation of nanoparticles might be a viable method to improve the bioavailability of drugs. Over the last few years several nano-formulations of essential oils have been reported each claiming to improve the bioavailability of the same. It has also been reported that use of polymeric nanomaterials improves the encapsulation of essential oils and eventually improve the bioavailability. These systems although suffer from several drawbacks, such as poor physical stability, drug leakage, and the potential toxicity of the excipients.[4]

Solid lipid nanoparticles (SLNs) have recently been under consideration for drug delivery because they offer the possibility of modulating drug release and provide both stability and compatibility while avoiding the shortcomings of liposomes, including undesired stability problems and the potential toxicity of the materials such as polymeric nanoparticles[5,6].

A few investigations have also been reported related to formulation of solid lipid nanoparticles using biocompatible and biodegradable lipid substances. Apart from physicochemical features, the intestinal permeability of a drug is another crucial factor for oral bioavailability[7-10].

It was there envisioned to use solid lipid nanoparticle approach as the carrier to improve the permeability of essential oils in the cells and hence the oral bioavailability. The lipids used to encapsulate essential oils into SLNs are likely to improve the aqueous dispersibility and stability of the essential oil, prolonging

its efficacy and cellular uptake and enhancing its bioavailability.

Material and Methods

All the reagents and chemical used in the present study were procured from various sources and used without any drying or purification. Thuja essential oil was procured from Veda oils, New Delhi, India.

Organoleptic characterization

A small quantity of Thuja oil was taken in a butter paper and viewed in well illuminated place to observe its color; the odor were observed by smelling the oil.

Solubility

Miscibility of Thuja oil was determined qualitatively in water, methanol and ethanol. Miscibility studies were performed by shaking small amount of the oil in test tubes containing the solvent and observing for layers (if any).

Boiling Point determination

The boiling point of Thuja oil was determined by placing the oil in a fusion tube and tying it to a thermometer. A capillary tube was dipped in the oil and the thermometer was dipped in Thiele's tube containing liquid paraffin. Heat was applied to the tube and the temperature at which boiling begins (indicated by bubble formation from side of the capillary tube) was recorded as the boiling point of the oil.

Refractive Index

The refractive index of the Thuja oil was determined by using Abbe's refractometer. The surface of the prism of refractometer was cleaned with acetone and a drop of oil was added on the lower prism. The lower prism was locked with the upper prism to form a film of the oil. Light was allowed to travel through the prisms and the change in path of the light was measured from the dial of the instrument.

Drug excipient compatibility Study

IR spectra of drug and a physical mixture of drug and lipids were obtained using FT-IR spectrophotometer. The spectra were observed for physical and chemical incompatibility amongst the drug and the lipids under study.

Calibration curve of Thuja oil

Stock solutions of Thuja oil containing 100 µg/mL were prepared in ethanol and its aliquots were transferred in a series of 10 mL volumetric flasks in varying fractions and their volumes were made with ethanol to prepare different standard dilutions (5-25 µg/mL). The solution was scanned using UV-Visible spectrophotometer from 1100 to 200 nm and the absorption maximum (λmax) was obtained to be 513 nm. The absorption of the standard dilutions was recorded at 513 nm to construct a calibration curve of concentration against absorbance. The linearity equation ($y = mx + c$) was generated and was used to calculate the concentration of Thuja oil in formulations.

Formulation of SLNs[11]

Probe-ultrasonication method was used for the preparation of the solid lipid nanoparticles. The organic phase was prepared by dissolving the lipid in a blend of 12 mL ethyl acetate and 8 mL ethanol (Table 1). Thuja oil was added to the organic phase and dissolved with stirring. A solution of Tween 80 was prepared in distilled water to obtain the emulsifier solution. The organic phase was drop wise added to the 2/3 of the aqueous phase with stirring (700 rpm) at room temperature and was sonicated using a probe sonicator for 5 min. The resultant pre-emulsion was dispersed in the remaining 1/3 of the aqueous phase kept in an ice bath. This

mixture was sonicated for 5/10 min under ice bath using probe sonicator (6 mm probe, 55% amplitude and 1 min pulses) to obtain the final lipid nanoparticles of Thuja oil (T-SLN).

Characterization of SLNs

Particle size and zeta potential determination[12]

Particle size was determined using a particle size analyzer while the zeta potential was determine using a zeta sizer. 1 mg/ml of nanoparticulate Thuja oil solution was prepared in double distilled water and sonicated for 30 seconds in an ice bath. 1 mL of this solution was diluted to 100 mL with deionized water and the particle size determination was done.

Entrapment Efficiency[12]

The percentage of drug incorporated during nanoparticle preparation was determined by centrifuging the drug loaded nanoparticles at 15,000 rpm for 15 min and separating the supernatant. The pellet obtained was washed twice with water and dissolved in acetonitrile followed by estimation of the drug by measuring the absorbance at 513 nm using UV-visible spectrophotometer.

$$\text{Entrapment Efficiency (\%)} = \frac{Amount\ of\ drug\ in\ nanoparticles}{Initial\ amount\ of\ drug\ taken} x100$$

Stability Study[12]

The stability of T-SLN was studied by storing at 4 ± 1 °C for 30 days. The particle size was observed to assess the physical stability of the SLNs while the drug concentration in SLNs was determined at the end of the study by spectrophotometry.

Antimicrobial action

Preparation of test solutions

The formulations were accurately weighed and dissolved in DMSO to prepare appropriate dilution to get required concentrations of 50, 100, 150 and 200 µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

Preparation of dried filter paper discs

Whatman filter paper (No.1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization

After sterilization, the discs were loaded with different concentration of broad spectrum antibiotic gentamicin and the test solution of different concentrations.

Application of Discs to Inoculated Agar Plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 h to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. The zone of inhibition was monitored after 24 h of incubation.

Results and Discussion

Physical characteristics

The results of organoleptic characterization and melting point are presented in Table 2. The result of solubility study revealed the miscibility of the Thuja oil in methanol and ethanol.

FT-IR study

The FT-IR spectrum of Thuja oil, and a physical mixture of Thuja oil, stearic acid were obtained and observed for any deletion of the peaks of the pure drug. The spectrum of Thuja oil exhibited peaks at 3341 cm^{-1} (OH stretching), 3056 cm^{-1} (CH aromatic stretching), 2923 cm^{-1} (CH_2

stretching), 1647 cm^{-1} (C=O stretching), 1574 cm^{-1} (C=C aromatic stretching), 1441 cm^{-1} (CH_2 bending), 1146 cm^{-1} (C-O stretching).

All the peaks were present in the physical mixture indicating a compatibility between the both the components.

Calibration curve of Thuja oil

The calibration curve of Thuja oil was prepared in methanol using UV-Visible spectrophotometer at 513 nm by plotting the absorbance against concentration (Figure 1). The linearity equation was found to be Absorbance (y) = 0.029 concentration (x) +0.005 with a regression coefficient value of 0.999 (R^2). This equation was used to calculate the concentration of Thuja oil in various stages of the study.

Preparation of T-SLNs

The preparation of the T-SLNs was achieved using probe-sonication method. Total of eight formulations of the T-SLNs were prepared by varying the concentration of the lipid, concentration of the surfactant and time of sonication and evaluated for particle size and entrapment efficiency.

The results of particle size and encapsulation efficiency obtained for all the formulated SLNs is presented in Table 3.

Effect of formulation variables on particles size

The particle size of the T-SLNs was found to usually increase with increase in concentration of the surfactant. This might be due to the surfactant-induced decrease in surface tension between the aqueous phase and organic phase. Besides, the surfactant helps to stabilize the fresh generated surfaces and avoids particle aggregation. On the other hand, the increase in concentration of lipid increased the particles size whereas increased sonication time were found to decrease the particles size of the SLNs. The particle size of the formulations ranged between 111 ± 2.88 to 126 ± 168.56 nm.

Effect of formulation variables on encapsulation efficiency

The results indicated that a change in lipid concentration had a higher impact on the encapsulation efficiency of the SLNs. Increasing the lipid concentration was found to increase the encapsulation of Thuja oil in the SLNs. This could be attributed to the fact that the lipid acts as a solubilizing medium of the lipophilic oil. The increasing concentration of surfactant also aided in improved solubility of the oil and hence increased the encapsulation efficiency. The encapsulation efficiency of the SLNs ranged between 71.23 ± 0.17 to 87.75 ± 0.30 %.

Effect of sonication time on particle size and encapsulation efficiency

The increase in sonication time was found to have a negative impact on the particle size and encapsulation efficiency. The formulation having the highest encapsulation efficiency and lowest particles size was found to be T-SLN3. This formulation was characterized for zeta potential, surface morphology, drug release and stability.

Characterization of T-SLN3

Particles size and zeta potential

The particle size and zeta potential of the formulation T-SLN3 were 125 ± 2.64 nm and -21.7 ± 2.61 mV respectively (Figure 2 & 3). The zeta potential value of around ± 30 mV is considered to be having stable particles. SLNs with zeta potential higher than 20 mV can be considered optimum for a formulation to be stable enough as a result of enough repulsion among

the particles that help in avoiding particle aggregation, making them stable for long term.

Stability Study

T-SLN3 was observed for change in particle size and encapsulation efficiency on storage at 4°C for a period of 30 days. The particle size remained stable at the end of the study with drug entrapment of 81.23%. This suggests that the SLNs prepared are stable on storage.

Antimicrobial activity

Disc diffusion method was used to assess the antibacterial action of the T-SLN3 against one gram positive and gram negative bacteria at four different concentrations (50-200 μM). The zone of inhibition was measured to confirm the antibacterial potential of the SLN (Table 4, Figure 4).

Conclusion

The aim of the present study was to prepare Thuja essential oil-loaded SLN to improve its bioavailability and drug loading. The results suggest that probe sonication method is a highly reasonable method for preparing the SLNs. The SLNs were evaluated for particle size, entrapment efficiency and antioxidant action. The particles were of smaller in size when high concentration of the lipid and surfactant was used and a lower sonication time was applied for preparation of the SLNs. The best formulation was one that was prepared with 2.0% stearic acid, 10.0% surfactant (Tween 80) and 10 min sonication.

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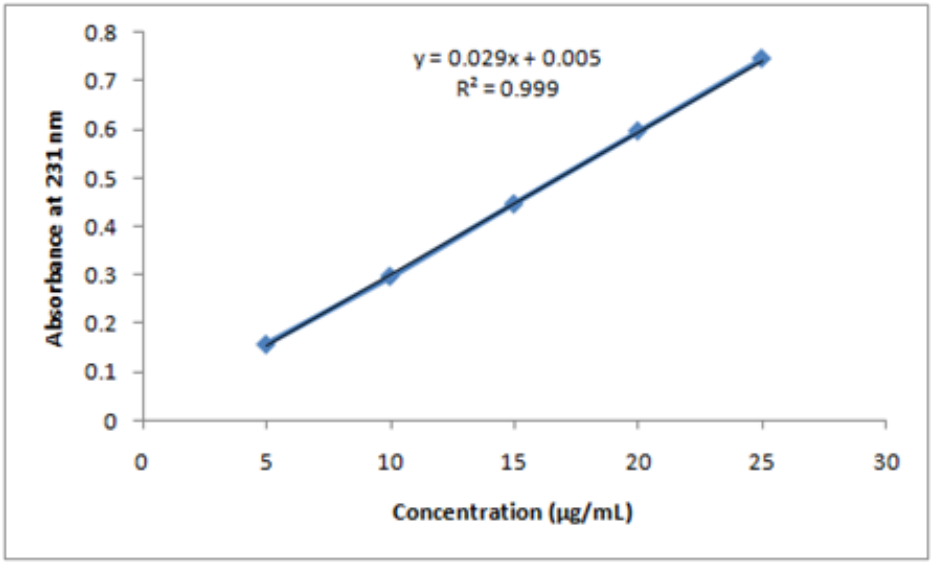

Figure 1 Calibration curve of Thuja oil in ethanol

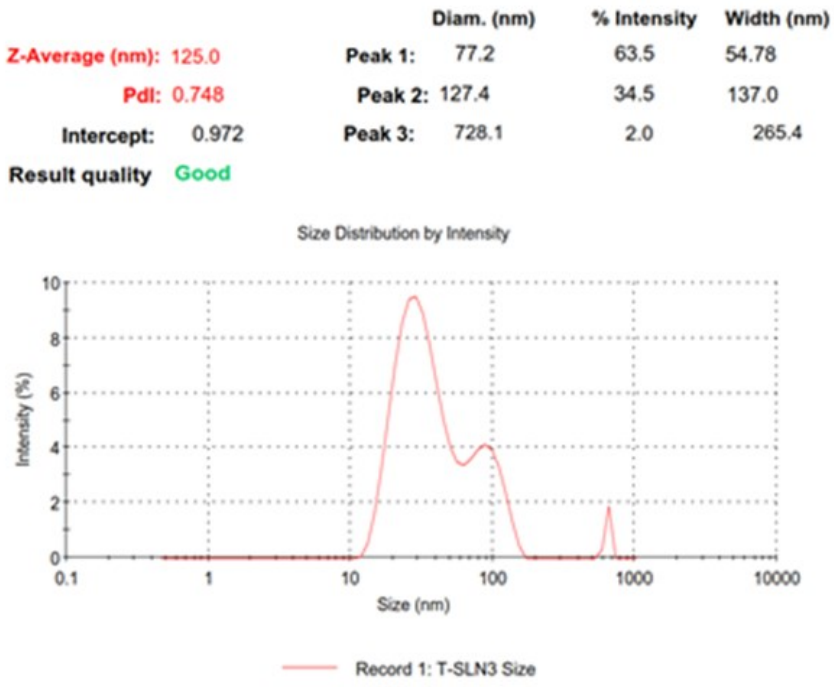

Figure 2 Particle size report of T-SLN3

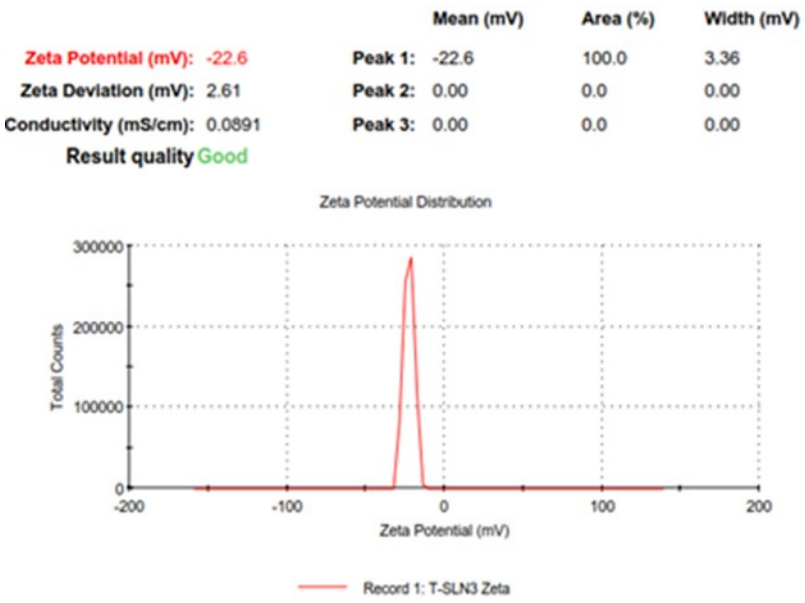

Figure 3 Zeta potential of T-SLN3

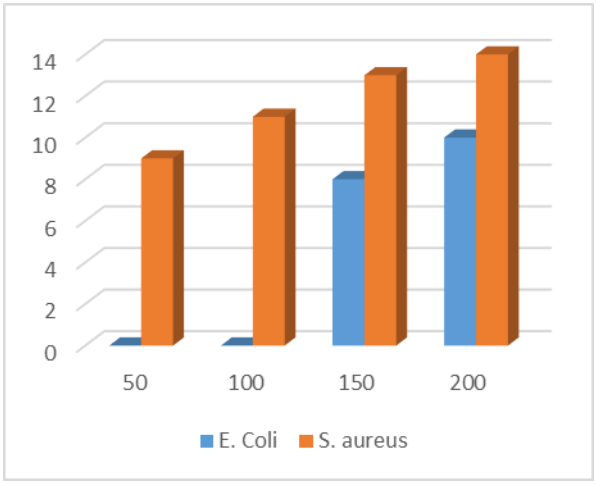

Figure 4 Antibacterial activity of T-SLN3

	T-SLN1	T-SLN2	T-SLN3	T-SLN4	T-SLN5	T-SLN6	T-SLN7	T-SLN8
Lavender oil (mg)	200	200	200	200	200	200	200	200
Stearic acid (%)	1.0	2.0	1.0	1.0	2.0	1.0	2.0	2.0
Tween 80 (%)	5.0	10.0	10.0	5.0	10.0	10.0	5.0	5.0
Soni-cationTime (min)	15	10	15	10	15	10	10	15

Table 1 Quantity of ingredients used as per design of experiment

Test	Specification	Observation
Color	Pale yellow	Light Yellow
Odor	Characteristic	Characteristic
Refractive Index	1.445-1.460	1.452
Boiling Point	113°C	115°C

Table 2 Organoleptic properties of Thuja oil

Run	Lipid con-centration (%)	Surfactant concentra-tion (%)	Soni-cation Time (min)	Particle size (nm)	Encapsula-tion Efficien-cy (%)
TSLN1	1	5	5	168 ± 5.56	71.23 ± 0.17
TSLN2	2	10	10	153 ± 3.0	80.11 ± 0.30
TSLN3	1	10	5	125 ± 2.64	82.92 ± 0.55
TSLN4	1	5	10	136 ± 2.64	74.67 ± 0.17
TSLN5	2	10	5	129 ± 1.73	83.18 ± 0.20
TSLN6	1	10	10	111 ± 2.88	80.59 ± 0.30
TSLN7	2	5	10	145 ± 3.51	87.75 ± 0.15
TSLN8	2	5	5	124 ± 2.00	83.32 ± 0.05

Table 3 Response obtained for optimization of T-SLNs

Concentration of T-	**Zone of Inhibition (mm)**	
	E. Coli	***S. aureus***
50	Nil	9
100	Nil	11
150	8	13
200	10	14

Table 4 Zone of inhibition of T-SLN3 against *E. coli* and *S. aureus*