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Development and evaluation of *Lepidium sativum* seed oil nanoemulsion

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Abstract

In the present study an attempt was made to formulate and assess the nanoemulsion formulation of *Lepidium sativum* Linn. (Garden Cress) seed oil (LSNE). *Lepidium sativum* seed oil contains many chemical constituents chiefly unsaturated fatty acids, α-tocopherol and have several pharmacological properties like antibacterial, antifungal, antioxidant, anti-inflammatory, and the oil is also responsible for relieving the pain in joints and rheumatism. Nanoemulsion was prepared by using non-ionic surface-active agent Tween 80, Span 20 and water by Ultrasonication technique. The average droplet size of the optimized nanoemulsion was determined as 208 nm. Evaluation of the nanoemulsion included analysis of particle size, polydispersity index, zeta potential, Ph, viscosity, refractive index and physical stability. The developed Nano formulation was found to be stable at long term stability study.

Keywords: *Lepidium sativum,* Nanoemulsion, Ultrasonication, Zeta potential, Seed oil

Introduction

Lepidium sativum L. (Cruciferae) is an annual, erect small edible herb cultivated as a vegetable crop all over India. The LS seeds are bitter, thermo genic, depurative, rubefacient, galactogogue, tonic, aphrodisiac, ophthalmic and diuretic [1]. The seeds hold an essential oil and fixed oil. Fixed oil has the benefits like; it is applied in rheumatic joints to relieve the pain and swelling [2]. The seed poultice is used to relieve the sprains, skin disease, dysentery, diarrhoea, dyspepsia, leucorrhoea, scurvy and seminal weakness [3]. LS seed oil possesses analgesic, antipyretic, anti-inflammatory, antiulcer [4], antihypertensive [5], antibacterial [6], diuretic [7], antiasthmatic [8], nephroprotective [9], hypoglycemic [10] and hepatoprotective [11] properties. The oil is the chief part of the plant which is responsible for several pharmacological and therapeutic applications. The LS seeds contain the fixed oil, α-tocopherol, carotenoids, saponins, amino acid, volatile oil and proteins The major fatty acid present in oil is linolenic acid (34%) followed by oleic acid (22%), linoleic acid (11.8%), palmitic acid (10.1%) and low amount of erucic acid and arachidonic acid are also present [12].

The Emulsions when reduce its droplet size (globule size) up to less than 100 nm becomes a stable system both thermodynamically as well kinetically, this system now called as an nanoemulsion. The NE system offers several benefits over conventional formulations. They have proved the transdermal permeation of many drugs. Nanoemulsions are developed systems for the delivery of biologically active agents for controlled release and drug delivery [13]. They are promising systems for the fields of cosmetics, diagnostics, drug therapy and biotechnology [14].

The present study was aimed at to formulate the o/w nanoemulsions of the extracted LS seed oil. The oil part is one of the phases of the biphasic system like emulsion. In our study oil itself acting as one phase of emulsion and also it will work for different pharmacological actions. The proposed nanoemulsion will offer all the benefits of a novel formulation as well as the versatility shown by the oil in its therapeutic uses. Our study is an attempt to explore the traditional knowledge with new modern concepts. Formulated nanoemulsion will contain with oil as the active ingredient with no other synthetic API in the formulation. Particle size, polydispersity index, zeta potential, Ph, viscosity, refractive index and physical stability are the other parameters to be evaluated for the LSNE formulation.

2. Materials and Methods

Materials

Lepidium sativum seeds were procured from local market of Satara, Maharashtra, India and authenticated at Dept. of Dravyaguna, Aryangla Vaidyak Mahavidyalaya, Satara.

The surfactant Tween 80 and Span 20 were purchased from Loba chemicals.

Extraction of *Lepidium sativum* seed powder

The shade dried seeds were powdered to appropriate size and about 200 g dried powder of seeds was extracted with 600 ml of Petroleum ether (40-60^{0}C) at temperature of 40-60^{0}C with a soxhlet apparatus for 7 hours. Then the extract was filtered and concentrated in a rotary evaporator till an oily extract was obtained. The percentage yield of the oil was calculated with respect to dry weight of the powder.

Physicochemical Evaluation of LS seed oil

The LS seed oil was evaluated as per the standard procedures for different physicochemical parameters like refractive index, acid value, Iodine value, saponification value, ester value, unsaponifiable matter and viscosity determination [15].

Preparation of LSNE

The o/w nanoemulsion of *Lepidium sativum* seed oil was prepared by using LS seed oil as dispersed phase in distilled water. The formulation was stabilized by the addition of the surfactant Tween 80 and co-surfactant Span 20. In order to get oil in water nanoemulsion Tween 80, a hydrophilic, non-toxic surfactant (HLB value 14.5) was chosen. Span 20 is having the HLB value (8.6). The combination of Tween 80 and Span 20 has been utilized in many nanoemulsion formulations and shown the stable system [16]. LSNE was composed of 10% of LS oil and various concentration ratios of surfactant and co-surfactant. Surfactant and co-surfactant concentrations were used as 1:0, 1:0.25, 1:0.5, 1:0.75, 1:1, 0:1, 0.25:1, 0.5:1, 0.75:1 etc. In this way, we got total 09 combinations of formulations and denoted as F1 to F9.In each formulation desired quantity of oil was thoroughly mixed with desired quantity of co-surfactant Span 20 using a mechanical stirrer at 500 rpm. Desired quantity of Surfactant Tween 80 was thoroughly mixed desired quantity of distilled water with gentle heat and constant stirring at 500 rpm. The water and surfactant mixture was subjected to ultra-sonication for 30 min using Ultrasonic processor (PCI Analytics, Mumbai), functions at 20 kHz with a highest power output 750 W. The oil and co-surfactant blend was added to the water and surfactant combination drop wise (6-7 drops/min.) by using a micro syringe. The details of the formulations are given in Table 1. To avoid extreme heating during sonication, ice bath was used to control the temperature.

Table 1: Composition of O/W LS seed oil Nanoemulsion formulations

Formulation	Concentration (%,w/w)					Appearance of Nanoemulsion
	LS Seed oil	Tween 80	Span 20	Ratio S to CS	Water	
F1	10	10	0	1:0	80	Milky
F2	10	10	2.50	1:0.25	77.5	Milky
F3	10	10	5.0	1:0.5	75	Translucent
F4	10	10	7.5	1:0.75	72.5	Transparent
F5	10	10	10	1:1	70	Translucent
F6	10	0	10	0:1	80	Milky
F7	10	2.5	10	0.25:1	77.5	Milky
F8	10	5.0	10	0.5:1	75	Translucent
F9	10	7.5	10	0.75:1	72.5	Translucent

Note- S=Surfactant CS=Co-surfactant

Characterization of LS Nanoemulsion

Centrifugation

The centrifugation of all the formulations was carried at 6,000 rpm for 30 min and the resistance of emulsion to centrifugation was studied. The formulations that didn't exhibit any phase separation were taken to further tests [17].

Heating cooling cycle

To study the thermodynamic stability of the formulations, this test was performed. The formulations (F1 to F9) were filled in glass ampoules and then subjected to heating and cooling cycle between 4°C (refrigerator temperature) and 45°C, each formulation being stored for 48 hours. The formulations were observed for phase separation and creaming after 48 hours. Those formulations, which were stable at these temperatures, were selected as optimized formulations and used for further study (Table 3) [17].

Viscosity

Viscosity is one of the important parameter for nanoemulsion. Selected optimized LSNEs were subjected to viscosity measurement. It was measured by using Brookfield viscometer (DV-II+Pro, USA) at 25˚C.Viscosity was determined in triplicate and the main purpose of this study was to establish rheological properties of formulations [18].

PH

Digital pH meter was used to determine the pH of the LSNE formulations. All the readings were taken in triplicate and the average was determined. To get a stable formulation pH is very important parameter and any change in pH may affect the stability of the formulation [19].

Refractive index

Abbe refractometer was used to determine the refractive index of the LSNEs by placing 1 drop of nanoemulsion on the slide at 25°C [20].

Droplet size analysis and Zeta Potential

The optimized LSNE formulations were subjected to droplet size measurement, determination of zeta potential and polydispersity index by using photon correlation spectroscopy and electrophoretic light scattering techniques by Nanoplus 3-Zeta/nano particle analyzer (Micromeritics, USA). Droplet sizes of the nanoemulsions are shown in Table 1. Deionised water was used as dilution medium and the measurements were performed at 25°C [13].

Stability of nanoemulsion

The stable, optimized LSNE formulations that have not shown any phase separation and creaming during the initial

Stability analysis (centrifugation and thermal tests) were subjected for long term stability testing. The formulations were filled in glass ampoules and then stored in stability chamber at different temperature conditions like 5 ± 2°C, 25 ± 2°C and 45 ± 2°C for 60 days. The little quantity of the sample was removed from each ampoule on day 10, 30 and 60 and was evaluated for physical changes. These were observed by visual observations for phase separation, creaming and change in appearance etc. The particle size and zeta potential were also recorded at above mentioned period by using Nanoplus 3-Zeta/nano particle analyzer.

3.0 Results and Discussion

% Yield of LS seed Oil

The percent yield of the extracted seed oil was determined with compared to dry weight of the seed powder, it was found to be 19%. We have used most non polar solvent (Petroleum ether) for extraction hence the yield was reasonable. The method of extraction was optimized by taking in to consideration the facts like temperature and time duration of extraction.

Physico-chemical characters of LS seed oil

In this, several parameters were studied; this evaluation of seed oils is an essential tool in the practice of quality control of seed oils. The authenticity of the seed oil and their deterioration is the big concern these days and their claim as unfit for human consumption could be saved by their proper evaluation before application. The results of physicochemical characters of LS seed oil have been shown in Table 2.

Table 2: Physico-chemical characterization of LS seed oil

S. No.	Parameter	Obtained values/Observations
1	Colour	Yellow brown
2	Odour	Pungent to characteristic
3	Taste	Bitter
4	Refractive index	1.48
5	Density	0.942gm/ml
6	Acid value	0.92
7	Iodine value	116.205
8	Saponification value	171
9	Unsaponifiable matter	0.527
9	Ester value	170.08
10	Specific gravity	0.91

Thermal stability of LSNE formulations

The stable formulations that survived the thermal stress and centrifugation only were selected for further analysis. Thermodynamically nanoemulsion is a stable system and is formed at a particular concentration of water, oil, surfactant and/or co-surfactant. From the observations F3, F4 and F9 nanoemulsions have survived the thermal stress and centrifugation tests. The details are shown in Table 3.The selected, thermodynamically stable LSNE formulations were subjected to further study.

Table 3: Thermal stability of LSNE formulations

Formulation	LS Seed oil	Tween 80	Span 20	Ratio S to CS	Water	Phase separation		Inference
						Cent.	H/C Cycle	
F1	10	10	0	1:0	80	Yes	--	Fail
F2	10	10	2.50	1:0.25	77.5	Yes	--	Fail
F3	10	10	5.0	1:0.5	75	No	No	Pass
F4	10	10	7.5	1:0.75	72.5	No	No	Pass
F5	10	10	10	1:1	70	No	Yes	Fail
F6	10	0	10	0:1	80	Yes	--	Fail
F7	10	2.5	10	0.25:1	77.5	No	Yes	Fail
F8	10	5.0	10	0.5:1	75	No	Yes	Fail
F9	10	7.5	10	0.75:1	72.5	No	No	Pass

Note-S=Surfactant, CS=Co-surfactant, H/C Cycle=Heating and cooling cycle, Cent. =Centrifugation

Droplet Size, Polydispersity index and Zeta potential Measurement

The mean droplet size, zeta potential and the polydispersity index were determined. The average droplet size of any nanoemulsion is between 50 to 1000 nm. The selected optimized LSNEs (Table 3) showed the mean droplet dimensions as F3-256±17 nm, F4-208±10 nm and F9-312±16 nm respectively (Table 4). Formulation F4 had shown minimum average particle size since it has the optimum concentration ratio of Tween 80 and Span 60 (1:0.75). Increase in Span 20 concentration from 5% to 7.5% decreases the mean particle size from 256 nm to 208 nm. Intentionally in all the LSNE formulations the concentration of oil was kept constant (10%) and the possible aim behind this was to study the behavior of the surfactant and co-surfactant ratio to get a stable nanoemulsion system. Polydispersity index (PI) determines the particle homogeneity and it varies from 0.0 to 1.0. If it is closer to zero then more homogenous are the particles. In our study the optimized formulations showed their PI in between 0.220 to 0.407, this designates suitable homogeneity [21]. The zeta potential value provides a sign of the possible stability of the colloidal system. A boundary between stable and unstable aqueous dispersion system is generally taken at either +30 or -30mV. Particles with zeta potentials more positive and negative +30mV and -30 mV are normally considered stable [22]. The optimized formulations (F3, F4 and F9) have shown zeta potential as -26 mV,-42mV and +32mV respectively which proves the formulations are significantly stable.

Viscosity determination

The optimized formulations have shown the values of viscosity are mentioned in (Table 4). Formulation F4 has the minimum viscosity (28.79 ± 2.13). Lower viscosity is an ideal characteristic of the o/w nanoemulsion.

PH Measurement

PH of all the formulations was found to be close to the value of skin pH (7.4).

Table 4: Droplet Size, Polydispersity index, Zeta potential, Viscosity, Refractive index and Ph Measurement of LSNEs

Formulation	Droplet size Mean± SD (nm)	Zeta Potential Mean± SD (Mv)	Polydispersity index (PI)	Viscosity Mean± SD (cP)	Refractive Index	pH
F3	256±17	-33.33±7.63	0.270	37.38± 2.66	1.35	7.46±0.55
F4	208±10	-40.52±7.0	0.220	28.79 ± 2.13	1.38	7.2±1.1
F9	312±16	-32.00±6.36	0.407	42.85± 2.64	1.54	6.8±1.3

Values represented as Mean ±SD (n=3)

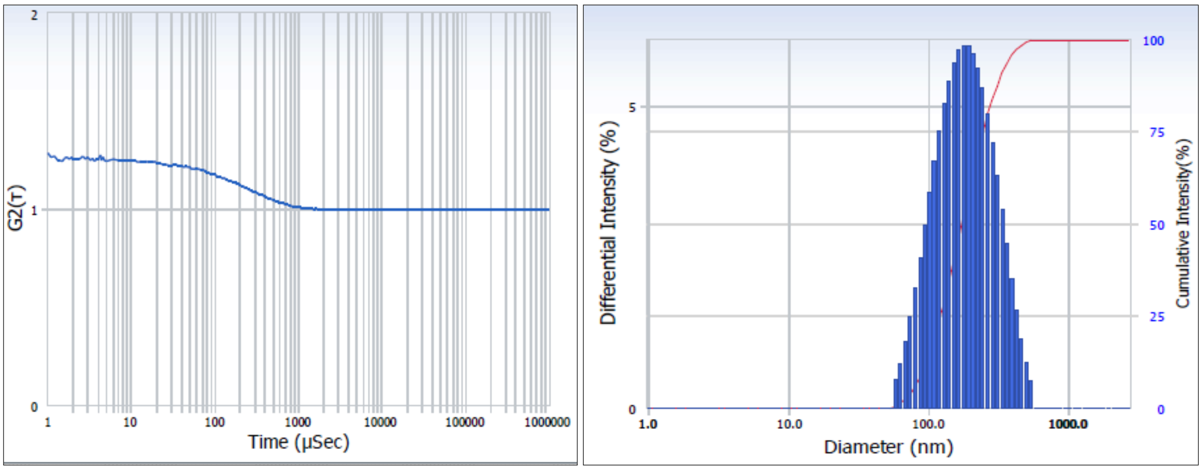

Fig 1: Mean Droplet size of formulation F4

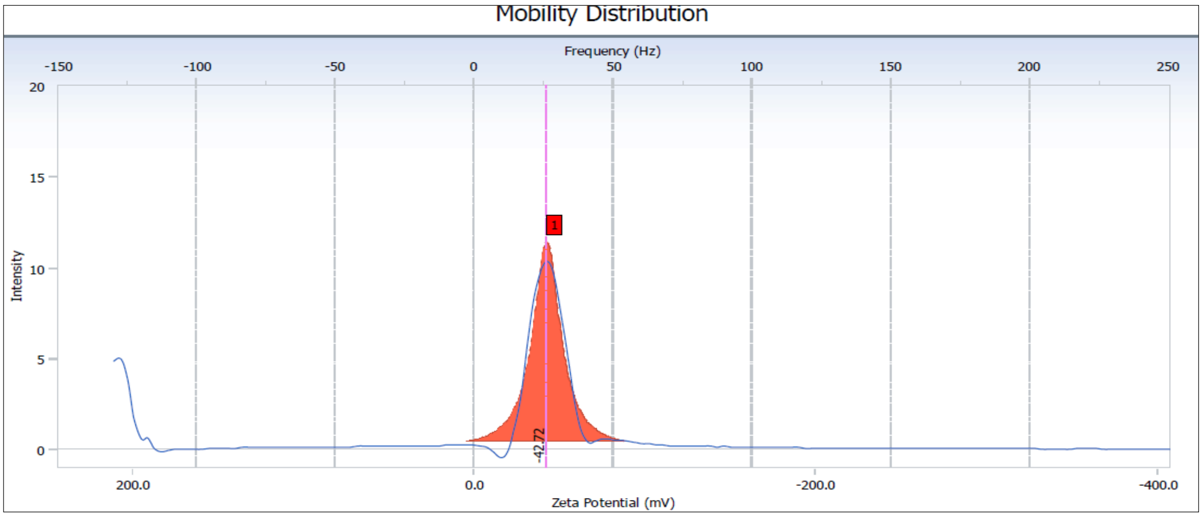

Fig 2: Zeta potential measurement of formulation F4 Stability of nanoemulsion

The stored formulations in stability chamber at different temperature conditions (5 ± 2°C, 25 ± 2°C and 40 ± 2°C) were observed for phase separation and any change in appearance upon standing for 60 days. The little quantity of the sample was removed from each ampoule on day 10, 30 and 60 and was evaluated for physical changes, particle size and zeta potential. The results are mentioned in Table-5.The results revealed that all the three formulation F3, F4 and F9 have shown no phase separation upon increase in temperature and did not show any significant change in mean particle size and zeta potential. Formulation F9 has shown the increase in mean particle size from 312 nm to 351nm. The probable reason behind this might be F9 has already possessed the greater particle size (312.17±16) compared with F3 (256.12±17) and F4 (208.0±10). It is recognized that the smaller droplet size will give better nanoemulsion stability; it is due to the fact that Brownian motion turn out to be more significant than the force of gravity. Assessment of droplet size and zeta potential are the most familiar technique taken in to consideration to evaluate the stability of nanoemulsions, since particle size hampers with flocculation and coalescence [23].

Table 5: Stability study of optimized LSNE formulations

Period of Evaluation	Droplet size in nm			Zeta Potential (mV)		
	F3	F4	F9	F3	F4	F9
After preparation	256.12±17	208.0±10	312.17±16	-33.33	-42.72	-32.00
After 10 Days	265.33±6.50	212.33±4.04	323.33±5.13	-23.48	-43.17	-35.21
After 30 Days	274.33±10.26	214.66±3.51	338.66±5.5o	-22.41	-41.62	-37.21
After 60Days	281.33±4.04	218.66±3.05	362.66±9.45	-21.20	-44.12	-19.16

4. Summary and Conclusions

On the grounds of greater stability, minimum droplet size, lowest polydispersity, suitable zeta potential value, low viscosity, optimum pH value and appropriate surfactant and co-surfactant concentration, formulation F4 was turn out to be better nanoemulsion of LS seed oil amongst all the others. The finding of this research is that it is possible to prepare a novel nanoformulation from traditional seed oil with a greater stability, so that it could be employed as a drug carrier for transdermal delivery. However, the seed oil itself is an important medicinal material of this plant and it could be explored as a part of novel drug delivery system.

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