International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 488-494
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

FORMULATION AND EVALUATION OF *PONGAMIA PINNATA* (KARANJ) SEED OIL IN WATER NANOEMULSION

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ABSTRACT

The present study was aimed to formulate and evaluate the Pongamia pinnata (Karanj) seed oil in water nanoemulsion by using low energy Emulsion Phase Inversion (EPI) method.To formulate the nanoemulsion, minimum concentration of non-ionic surfactant Tween 80 and Span 20 were used. The nanoemulsions were evaluated for analysis of globule size, polydispersity index, zeta potential, pH, viscosity, refractive index and physical stability. The optimized nanoemulsion was composed of 6% Karanj oil, 40% Smix and 54% aquous phase. The average droplet size of the optimized nanoemulsion was found to be 245 nm. The results revealed that, the developed nanoemulsion was found to be stable at long term stability study.

KEY WORDS

Pongamia pinnata, Nanoemulsion, Zeta potential, Seed oil

INTRODUCTION

Oil-in-water emulsions are essential vehicles for the transportation of lipophilic active drugs by enhancing its absorption through skin, improved retention time of the drug in the objective region and ending in fewer side effects. Nanoemulsions (NEs) are being employed in a large amount in the commercial areas like the pharmaceutical, chemical and cosmetic industries [1]. NEs are having the globule size reached approximately to 20-500 nm. The small globule size can improve the physical stability which is generally being hampered due to gravitational separation, flocculation and/or coalescence. It also prevents the creaming process since the gravitational separation forces are comparatively not enough than droplet's Brownian motion [1]. NEs improved the pharmacological effects of the drugs adopted, have reduced drug side effects and incorporated in intravenous, oral, ocular and transdermal drug administrations. [1]. NEs are principally formulated either by high-energy (high-pressure homogenization, ultra-sonication) and low energy method (spontaneous emulsification (SE) method, phase inversion temperature (PIT) method, phase inversion concentration (PIC) method and emulsion inversion point (EIP) method) [2].

Pongamia pinnata (L.) Pierre (Leguminosae) is a significant non-edible seed oil plant. Oil is the most notable product obtained from the seeds which is a thick, yellow to reddish-brown in colour [3]. Karanj seed has been used as a medicinal plant since centuries in Ayurvedic and Siddha medicine systems of India. Additionally, the oil has many commercial applications as a lubricant, as water-paint binder, in leather dressing and in soap-making [4].

Karanj is the most important biofuel plant used as medicinal plant too. Regardless of the controversy about toxic components like flavonoid and erucic acid, the seed of this plant has been employed in traditional medicine and its oil is used in Ayurveda to treat psoriasis and arthritis. The seed extracts have exhibited several pharmacological properties like antibacterial, antifungal, antiviral, anti-ulcer, anti-inflammatory [5] and anti-arthritic [3].

Karanj oil has many applications for skin and skin ailments. So, in the present study we have prepared the karanj seed oil nanoemulsion that would offer all the benefits of the novel drug delivery system. If this formulation has been employed as a transdermal delivery, then it might be producing synergistic effects since oil itself has the important bioactive molecules like Karanjin and pongamol with important biological characteristics. Several studies have revealed that both (Karanjin and pongamol) are pharmacologically active for inflammation, arthritis and skin ailments [3, 5].

MATERIALS AND METHODS

Materials

Pongamia pinnata (L.) 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 60 were purchased from Loba chemicals.

Extraction of *Pongamia pinnata* seed oil

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 40-60^{0}C with a soxhlet apparatus for 7 hours [6]. Then the extract was filtered and concentrated in a rotary evaporator till an oily extract was obtained. The percent yield of the oil was determined with respect to dry weight of the powder.

Physicochemical Evaluation of Karanj seed oil

The Karanj seed oil was evaluated as per the standard procedures for different physicochemical parameters like refractive index (RI), acid value, Iodine value, saponification value, ester value, and unsaponifiable matter [7].

Construction of Phase Diagram

For the development of karanj oil NE, Tween 80 (HLB 15) was selected as surfactant and Span 20 as co-surfactant. Tween 80 is a food grade, non-ionic, safe, non-irritant surfactant generally been used for the preparation of o/w emulsions [8]. Span 20 is lipophilic surfactant having HLB 8.6. Three different weight ratios (1:0, 1:1 and 1:2 w/w) of surfactant to co-surfactant (Smix) were prepared to achieve optimum ratio which can result in maximum nanoemulsion existence area. Phase diagrams were constructed by mixing karanj oil and specific Smix ratio thoroughly in different volume ratios from 1:9 to 9:1 v/v in different glass containers. Total 17 such different combinations of oil and Smix were obtained (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1,3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1). For each Smix-oil combination separate phase diagram was constructed. Aquous phase was added slowly in each combination by titration method. In each oil- Smix combination the percentage of oil and Smix was determined with respect to the volume of water added to each combination. The quantity of water phase added was different to produce a different water concentration in every combination (Oil-Smix). In the process of water addition to the oil-Smix combination, we have observed different morphological nature of the nanoemulsions viz. milky or cloudy emulsion, thick cloudy emulsion, translucent emulsion and transparent to easily flowing o/w nanoemulsions. Different phase diagrams have been demonstrated in Fig.1 [9].

Formulation of Karanj Oil Nanoemulsions

By virtue of pseudoternary phase diagrams, various ratios of Smix and oil were removed out and then desirable quantity of water was mixed to the mixture drop wise. Further the nanoemulsion was obtained by continuous stirring at 600 rpm on a magnetic shaker at room temperature. The prepared formulations were stored at room temperature till further use.

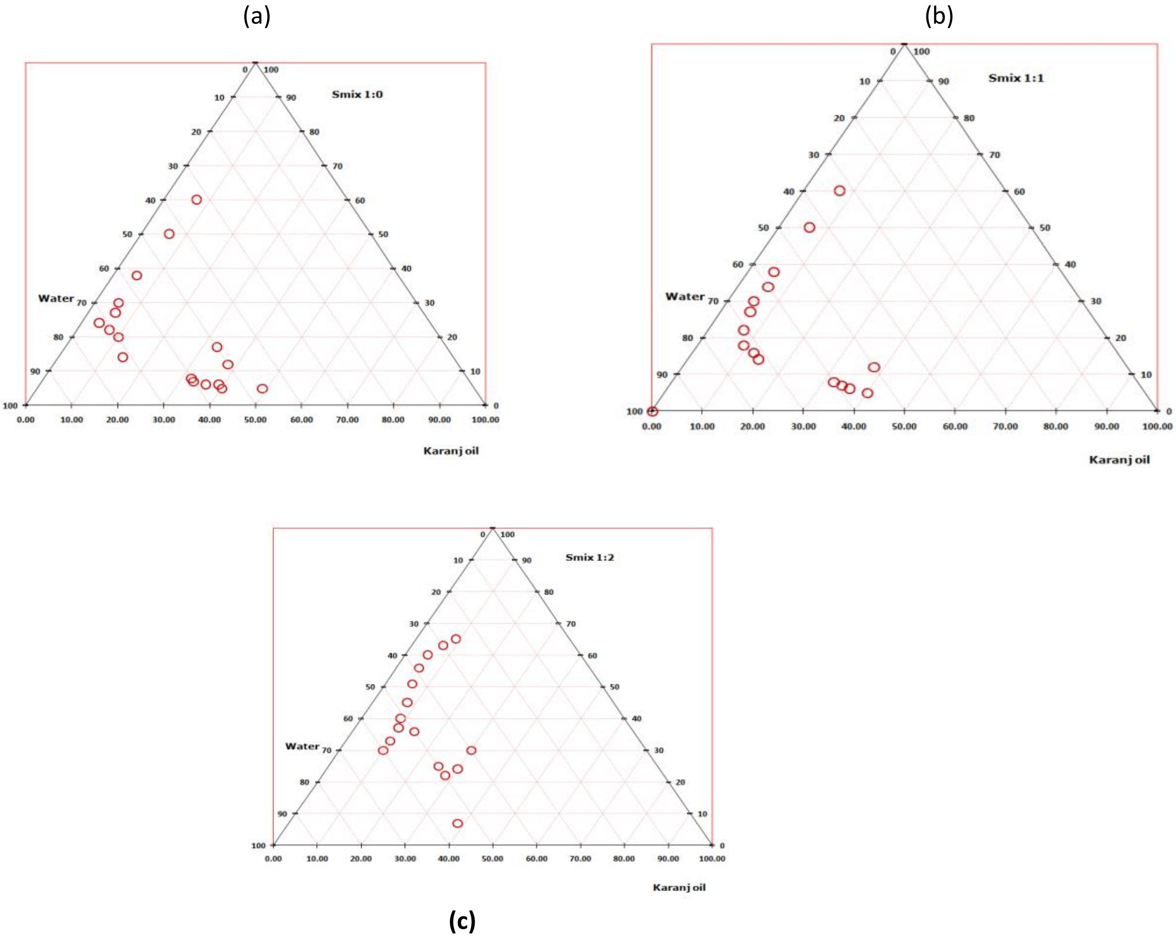

Fig.1 Pseudoternary phase diagram denoting Nanoemulsion region at different Smix ratios a-(1:0), b-(1:1) and c-(1:2)

Characterization of Karanj Oil Nanoemulsions

Determination of Viscosity and pH

Viscosity 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 [10].

pH of the nanoemulsions was determined by using Digital pH meter at 25 ± 1°C. 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 [11].

Refractive index

Refractive index of all the selected nanoformulations was determined by using Abbe refractometer by placing 1 drop of nanoemulsion on the slide at 25°C [12].

Zeta Potential and Droplet size analysis

The selected Karanj Oil Nanoemulsions were screened to measure droplet size, to determine zeta potential and polydispersity index (PDI) by using photon correlation spectroscopy and electrophoretic light scattering techniques by Nanoplus 3-Zeta/nano particle analyzer (Micromeritics, USA). The results have been shown Table 2. Since all are the o/w nanoemulsions, double distilled water was used as diluents and the measurements were carried out at 25°C [13]

Stability of nanoemulsions

The optimized karanj oil nanoemulsion was filled in glass ampoules and then stored at different temperature conditions like 4°C and 25°C for 90 days. The little quantity of the sample was removed from each ampoule on day 1, 30, 60 and 90 was evaluated for physical changes, phase separation, creaming and change in appearance etc. These were observed by visual observations for phase separation. The particle size and zeta potential were also recorded at above mentioned period by using Nanoplus 3-Zeta/nano particle analyzer [14].

RESULTS AND DISCUSSION

Percent Yield of Karanj seed oil

To get the optimum yield of the extract from plant material, the choice of extraction method, solvent and duration of extraction are essential parameters. In view of this, the method of extraction was optimized by considering above facts. The percent yield of the extracted seed oil was found to be 28% and was determined with compared to dry weight of the seed powder.

Physico-chemical characters of Karanj seed oil

The optimum values of the physico-chemical characters give an idea about the purity and authenticity of the seed oil. In this, several parameters were studied and the results of physico-chemical characters of karanj seed oil have been shown in Table 1.

Table 1-Physico-chemical characterization of Karanj seed oil

S.No.	Parameter	Obtained values/Observations
1	Colour	Dark brown
2	Odour	Pungent to characteristic
3	Taste	Bitter
4	Refractive index	1.47
5	Density	0.932 g/ml
6	Acid value	1.77
7	Iodine value	78
8	Saponification value	180
9	Unsaponifiable matter	3
10	Specific gravity	0.93

Selection of Nanoemulsion formulations

The basic purpose to plot a pseudoternary phase diagram is to locate the existence range of nanoemulsions. In the phase diagrams of our study the translucent to transparent zone of nanoemulsion has been presented. The remaining region exhibits the turbid/milky emulsion zone. In our study three different weight ratios (1:0, 1:1 and 1:2) of surfactant to co-surfactant (Smix) were prepared to achieve optimum ratio. Pseudoternary phase diagrams were constructed separately for each surfactant to cosurfactant ratio, so that o/w nanoemulsion regions could be identified. From the different phase diagrams it is revealed that the optimum nanoemulsion region has been exhibited by the surfactant to co-surfactant ratio1:1(w/w).As seen in other ratios of Smix (1:0 and 1:2 w/w), we have observed the turbid to milky thick emulsions. In Smix ratio 1:2, the amount of co-surfactant Span 20 is greater so that aggregation of the system occurred since it is a lipophilic surfactant. Hence the Smix ratio 1:1 (w/w) was optimized and seven nanoemulsions from this ratio were selected randomly for further study. The percent values of all the components (oil, Smix and water) for the selected nanoemulsions have been presented in Table 2 along with other details.

Droplet Size, Polydispersity index, Zeta potential of selected nanoemulsions

The mean droplet size mean zeta potential and polydispersity index of all the seven selected formulations have been presented in Table 2. The nanoemulsion has the droplet size up to 500 nm. In the observations, F2 nanoemulsion has shown minimum mean droplet size (245 ±7.23 nm) with polydispersity index 0.194 compared with others. It also reveals that as the concentration of oil has enhanced as in F4 to F7, the mean droplet size has also been increased. F2 formulation contains minimum concentration of oil (6%) and also considerable amount of Smix (40%). Near to F2 nanoemulsion, F3 has also shown considerable results as mean droplet size 247.66±3.05 nm. with polydispersity index 0.194. Zeta potential is the measure of positive or negative charges on the globule. This charge should be strong enough to make the globules to repel each other so that there would not be the coalescence of the droplets and the emulsion would break. The zeta potential value provides a sign of the possible stability of the colloidal system. Particles with zeta potentials more positive and negative (+30mV and -30mV) are normally considered stable [15]. In this study; the zeta potential values reveal that the formulations are stable since they all exhibit the values more than -30 mV.

Table 2- Droplet Size, Polydispersity index, Zeta potential of selected nanoemulsions

Formulation	Composition % w/w			Droplet size Mean± SD (nm)	Zeta Potential (mV)	Polydispersity index (PI)
	Oil	Smix 1:1(w/w)	Water			
F1	6	37	57	249.65±3.53	-37.33	0.195
F2	6	40	54	245.0±7.23	-38.14	0.194
F3	7	43	50	247.66±3.05	-34.00	0.194
F4	10	45	45	260.99±6.16	-42.00	0.195
F5	10	40	50	255.58±10.9	-39.33	0.194
F6	15	45	40	260.74±5.98	-42.33	0.199
F7	15	40	45	262.04±5.16	-43.66	0.191

Values represented as Mean ±SD (n=3)

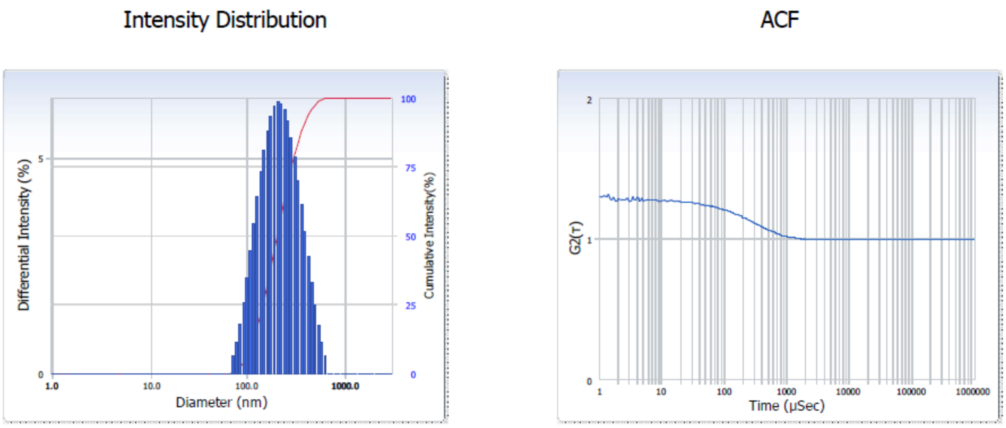

Fig.-2 Droplet size and size distribution of optimized nanoemulsion F2

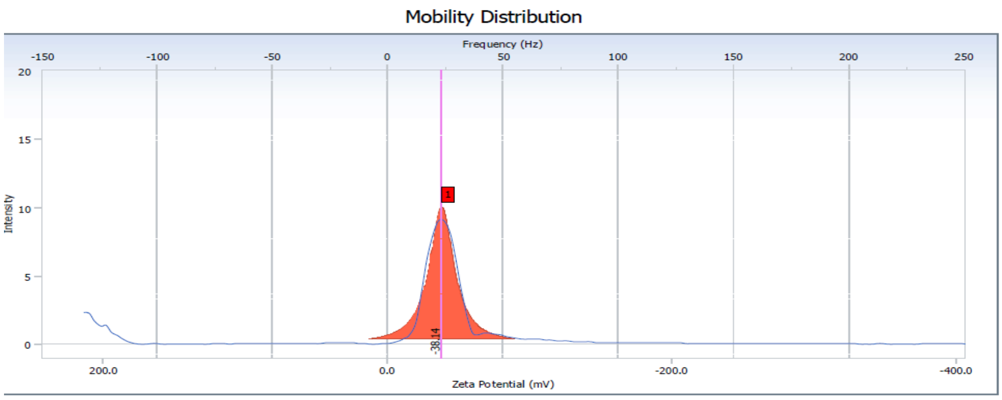

Figure-3 Zeta potential measurement of nanoemulsion F2

Determination of Viscosity

The results of viscosity revealed that viscosity likely to increase with the increase in the oil concentration [14]. The oil content was increased from 6% w/w to 15% w/w, also the increase in the viscosity of the nanoemulsions was observed, the results are mentioned in Table 3. The viscosity of formulation F2 and F3 was considerably low compared with other formulations since they contain low oil content. Considering as a whole, all the selected formulations have exhibited low viscosity which is a sign of nanoemulsion formulation.

pH Measurements

The pH of all the selected formulations was measured in triplicate by digital pH meter at 25 ± 1 °C. The values were found in between 6-8. pH of all the formulations was found to be close to the value of skin pH (7.4). The said pH range is most favorable for transdermal nanoemulsion.

Refractive index

Refractive index (RI) of selected nanoemulsions was determined by using an Abbe refractrometer. The results reveal that as the concentration of the oil increases in the formulation, the refractive index also increases. Formulations F2, F3 have presented a low refractive index. Also, it is noticed that as the particle size increases, the RI increases, in formulation F5, F6, F7 it was noticed since they have a larger particle size.

Table-3 Viscosity, pH and Refractive index of the nanoemulsions

Formulation	Viscosity Mean± SD (cP)	Refractive Index	pH
F1	40.33±3.05	1.35±0.045	6.66±0.32
F2	39.66±4.93	1.34±0.045	6.83±0.15
F3	42.33±4.16	1.37±0.037	7.36±0.40
F4	52.0±3.0	1.39±0.032	6.9±0.1
F5	55.33±4.04	1.42±0.025	6.73±0.15
F6	56.33±3.21	1.45±0.040	6.80±0.16
F7	39.66±4.93	1.43±0.017	6.83±0.20

Values represented as Mean ±SD (n=3)

Stability studies

The primary intention of stability testing is to check the quality of the formulation upon long term standing and also to check the effects of various environmental factors such as temperature, humidity and light on the formulation. The stabilized formulation is one that stands fit physically, chemically and microbiologically. From the observations and results obtained so far, we have confirmed F2 nanoemulsion formulation as optimized formulation. Optimized Karanj Oil Nanoemulsion (F2) was evaluated at different temperature conditions like 4°C and 25°C for 90 days. To check the stability, the formulation was visually evaluated for physical changes, phase separation, creaming and change in appearance etc. Optimized nanoemulsion formulation (F2) was examined for droplet size and zeta potential at 25 °C on 1, 30, 60 and 90 days of the study. The results revealed that there is marginal increase in the droplet size and zeta potential at the selected temperature (25 °C). The mean droplet size of F2 was found to be 258.07±2.68 nm after 90 days. When compared with initial value, it is increased marginally. The probable reason might be decreased repulsion between the particles to prevent the coalescence of the particles Zeta potential of F2 formulation on day 90 was decreased (from -38.14 to -32.00). There was not any phase separation observed during the course of the study at different temperatures. The results of the stability testing have been presented in Table -4.

Table-4 Stability study of optimized Karanj Oil Nanoemulsion

Period of Evaluation	Droplet size in nm	Zeta Potential (mV)
	F2	F2
After preparation (25 °C)	245.0±7.23	-38.14
After 30 Days (25 °C)	252.2±2.86	-37.26
After 60 Days (25 °C)	254.93±4.43	-33.84
After 90 Days (25 °C)	258.07±2.68	-32.00

Values represented as Mean ±SD (n=3)

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 F2 was turned out to be better nanoemulsion of Karanj seed oil amongst all the others. Karanj seed oil has many benefits as far as skin is concerned, so the formulated nanoemulsion could be a better option to use as a carrier for other synthetic drugs which have similar action as that of karanj. It is also possible to get the synergistic effect by virtue of karanj NE and loaded synthetic drug in NE. 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.

ACKNOWLEDGEMENT

The authors are thankful to Gourishankar Education Society's Satara College of Pharmacy, Satara and Department of Pharmaceutics, H.R. Patel college of Pharmacy, Shirpur for providing all the research facilities and support.

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Received:06.08.18, Accepted: 09.09.18, Published:01.10.2018

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