FORMULATION DEVELOPMENT AND IN VITRO ANTIOXIDANT AND ANTIDIABETIC EVALUATION OF ERIobotryA JAPONICA BASED SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

Objective: The aim of the present study was to develop and characterize self-nano emulsifying drug delivery system (SNEDDS) of methanolic extract of Eriobotrya japonica (Thunb.) Lindl. (E. japonica) leaves. Further in vitro antioxidant and antidiabetic potential of an optimized batch of SNEDDS was explored.

Methods: Oil (Labrafil M 1944 CS), surfactant (Tween 80) and co-surfactant (Transcutol P) were selected on the basis of solubility of the methanolic extract. Twenty-seven batches of SNEDDS were prepared with different compositions of oil, surfactant and co-surfactant. The optimized batch was evaluated for its entrapment efficiency, droplet size, polydispersity index (PDI), zeta potential, transmission electron microscopy (TEM). Further, DPPH assay and α-amylase activity were also performed to check the antioxidant and antidiabetic potential of prepared SNEDDS.

Results: The optimized design suggested that 10% of Labrafil M 1944 CS, 30% of Tween 80 and 60% of Transcutol P could develop SNEDDS with 208 nm mean droplet size, 99.64% drug loading, 0.156 PDI and -6 mV zeta potential. TEM image confirmed the droplet size less than 100 nm and the spherical shape of SNEDDS. In vitro antioxidant and antidiabetic activities of SNEDDS revealed the increased efficacy as compared to that of the ascorbic acid and acarbose, respectively.

Conclusion: The optimized batch of SNEDDS was found to improve the antioxidant and antidiabetic efficacy of methanolic extract of E. japonica.

Keywords: SNEDDS, Eriobotrya japonica, DPPH, Antidiabetic, Antioxidant

INTRODUCTION

Ethnomedicine is the traditional medical practice of indigenous culture that gave remedial and palliative effects of a complex multi-disciplinary system constituting the use of herbs, spirituality and the natural environment, which have the ability to promote the health and healing potential for humanity. Efficacy of ethnomedicines mainly depends on the presence of pharmacologically active phytoconstituents. Moreover, development in the fields of phytochemistry and phytopharmacology has increased exploration of ethnomedicines for their effective biological attributes [1-4]. However, these phytoconstituents are not permeable to the lipid membranes due to the high molecular weight and low rate of absorption, which ultimately affect the bioavailability and efficacy of the drugs. Apart from this, various drawbacks related to the dose, palatability, stability, and toxicity of the phytoconstituents and their formulations have also been reported. Therefore, recent studies based on the nanotechnology and nanopharmaceuticals have suggested the integration of phytoconstituents and nanotechnology because nanocarriers can efficiently carry such phytoconstituents at the desired site of action inadequate concentration and can be helpful to address the associated drawbacks of the phytochemicals [5].

Recently, the development of self-nano emulsifying drug delivery system (SNEDDS) for herbal drugs has received a lot of attention as a novel approach to overcome drawbacks associated with plant-based medicines. The SNEDDS is isotropic and thermodynamically stable mixtures of oil, surfactant (HLB=12) and co-surfactant. The SNEDDS can emulsify spontaneously in situ with the contact of gastrointestinal tract (GIT) fluid. The SNEDDS can be an effective alternative for the plant extracts by taking the benefit of lipophilic nature to overcome their drawbacks related to the poor solubility, bioavailability, absorption, palatability, and instability [5].

Eriobotrya japonica (Thunb.) Lindl. (E. japonica), a traditional medicinal plant used to treat diverse pathophysiological conditions is also consumed as a food product in daily life. Generally, it is used in East Asian countries like Japan, China, Korea, India, Nepal, and Pakistan. In India, it is mainly used in various states including Uttar Pradesh, Punjab, and Himachal Pradesh. The traditional healers and Vaidyas use the juice of E. japonica leaves to treat diabetes. The recent studies suggested the diversified pharmacological effects of E. japonica including antidiabetic effect [6], neuroprotection [7], cardiovascular health [8], glucose metabolism [9], anti-obesity [10], bone and joint strength [11], anti-inflammatory [12], hormonal activity [13], effects on peripheral organ systems [14] and anticancer activities [15].

The present study was designed to develop and characterize the SNEDDS based ethnomedicine and explore the improved antioxidant and antidiabetic potential of E. japonica.

MATERIALS AND METHODS

Materials

Labrafil M 1944 CS, and Transcutol P were received from the Gattefosse, Mumbai, India as gift samples. Tween 80 and Ethanol were purchased from Loba Chemie (P) Ltd., Mumbai (Maharashtra), India. The double distilled water of USP grade was used throughout the study. The fresh leaves of E. japonica were collected from village Kotla Naudh Singh of district Hoshiarpur (Punjab). The latitude 31.5977701 and longitude 75.8330591 are the geocoordinates of Kotla Naudh Singh village. The leaf sample of E. japonica was authenticated from National Institute of Pharmaceutical Education and Research (NIPER), Mohali with NIPER/2018/AS/3 voucher specimen number.

Preparation of methanolic extract of E. japonica

Leaves of E. japonica were washed with running tap water and shade dried. Coarse powder of shade-dried leaves was prepared for the extraction using Soxhlet apparatus. The coarse powder (25 g)
was kept in a thimble and 150 ml of methanol as a solvent was used for the extraction. The process was continued for 24 h at 65 °C. The extraction was repeated six times. After the continuous extraction process, methanol was evaporated by using a water bath to get the dried extract [16].

UV spectroscopic analysis of the extract

The dried extract (300 mg) was dissolved in 6 ml of ethanol for the preparation of stock solution having a concentration of 50 mg/ml. Solutions with different concentrations ranging from 10, 20, 30, 40 and 50 µg/ml were prepared from the stock solution by appropriate dilutions and analyzed spectrophotometrically (1601 UV-vis spectrophotometer, Shimadzu, Kyoto, Japan) in the wavelength range of 200-400 nm. The absorbance of standard solutions was measured at 275 nm 

Solubility studies

For the preparation of SNEDDS, the solubility study was performed using oil (Labrafil M 1944 CS), surfactant (Tween 80) and co-surfactant (Transcutol P) (Table 2). A briefly predetermined amount of extract was mixed in oil surfactant and co-surfactant separately, vortexed for 15 min and then kept shaken for 3 d on a water bath at 25 °C. The resulting mixtures were centrifuged at 3500 rpm for 15 min. The supernatants were collected and analyzed by UV-Visible spectrophotometer at 275 nm. The study was repeated in triplicate and mean data was recorded.

Preparation of self-nano emulsifying drug delivery system

On the basis of solubility of the extract, the oil (Labrafil M 1944 CS), surfactant (Tween 80) and co-surfactant (Transcutol P) were selected and 27 formulations of SNEDDS were prepared by selecting different concentrations of the oil, surfactant and co-surfactant ranging from 10-70%. Each composition was loaded with 150 mg of extract. The resultant mixture was sonicated by using ultrasonicator for 30 min at 37 °C. Further, the isotropic mixture was diluted with 250 ml of double distilled water and stirred at 100 rpm at 37 °C using the magnetic stirrer to check the emulsification of the selected oil, surfactant, and co-surfactant. The prepared emulsions were visually evaluated for their relative turbidity. The formation of proper nanoemulsion was determined when uniform dispersion of droplets into the water resulted in transparent milky emulsion whereas immediate coagulation of droplets after stirring resulted in turbid emulsions. The formation of nanoemulsion was determined spectrophotometrically at 275 nm. The absorption of standard solutions was measured at 275 nm \( \lambda_{\text{max}} \) and a calibration curve was plotted.

Preparation of self-nano emulsifying drug delivery system

Table 1: Observation table of extraction of leaves of E. japonica

| Observations           | Batch | I   | II  | III | IV  | V   | VI  | Average |
|------------------------|-------|-----|-----|-----|-----|-----|-----|---------|
| Weight of drug (g)     |       | 25  | 25  | 25  | 25  | 25  | 25  | 25      |
| Volume of solvent (mL) |       | 150 | 150 | 150 | 150 | 150 | 150 | 150     |
| Yield of extract (%)   |       | 12  | 9.6 | 9.6 | 11.2| 11.2| 12.8| 11.06   |
| Duration (hours)       |       | 24  | 24  | 24  | 24  | 24  | 24  | 24      |

% Entrainment Efficiency = \( \frac{\text{Amount of encapsulated drug}}{\text{Amount of total drug}} \times 100 \)

Droplet size and Zeta potential analysis

The average droplet size, polydispersity index (PDI) and zeta potential of the optimized batch of SNEDDS were measured by Zetasizer Nano zetasizer instrument (DTS Ver. 5. 10). The optimized batch of SNEDDS (0.1 ml) was dispersed in 100 ml of water. Further, 1 ml of the aliquot was introduced into a sample cell for the measurement of the droplet size [18,20]. The temperature was maintained at 25 °C and the angle was fixed angle at 90° during the evaluation.

Transmission electron microscopy

The size of droplets of the prepared formulation was determined by Transmission electron microscope (H-600, Hitachi, Japan) under 20000X resolution. Briefly, for the preparation of the sample, an optimized batch of the prepared formulations was diluted with 1000 ml of water to prepare an emulsion. Further, it was stained using 2% w/v phosphotungstic acid and kept on the copper grids of 400-mesh for films for evaluation [20, 21].

In vitro antioxidant activity

The antioxidant activity was determined by the DPPH assay method [22]. Briefly, 200 µl of the analytical sample and 800 µl of 0.1M Tris-HCl Buffer (pH 7.4) were added in a test tube. Ethanolic DPPH solution (1 ml) was added immediately and test tubes were shaken for 10 seconds. Then, the solution was kept in a dark place at room temperature for 30 min. The absorbance of the solution was measured at 517 nm [22]. The study was repeated in triplicate. Percentage of inhibition was calculated by using the following formula:

\[
\text{Percentage inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

In vitro α-amylase inhibition assay

The α-amylase (500 µl) was added into different dilutions (0.075-0.75 mg/ml) of the sample and standard drug. After incubation at 25 °C for 10 min, 500 µl of 1% starch solution was added. Again, it was incubated at 25 °C for 10 min. In the next step, 1 ml DNS reagent was added and kept in a boiling water bath for 5 min. After the self-cooling, add 10 ml of distilled water in each test tube and the absorbance was taken at 540 nm [22]. The percentage of inhibition was calculated by using the following formula:

\[
\text{% Enzyme activity} = \left( \frac{[A - C] - [T]}{A - C} \right) \times 100
\]

Where, A, C, and T were the absorbance of negative control, positive control and test sample, respectively.

RESULTS AND DISCUSSION

Extraction of E. japonica

The methanolic extract of dried leaves of E. japonica was obtained with the help of the Soxhlet apparatus. The percentage of yields of different batches were found to be 12, 9.6, 9.6, 11.2, 11.2 and 12.8%, respectively. The average yield of extract was 11.06% (Table 1).
UV spectroscopic analysis

The concentration of the extract was taken 1 mg/ml for UV spectroscopic determination of the \( \lambda_{\text{max}} \), which was observed as 275 nm. It was used to determine the linearity of a serial dilution of the extract. The observed absorbance of UV spectra with respect to different concentration (10-50 µg/ml) was plotted as the standard graph. The observed linear regression equation was found to be \( Y = 0.0288x + 0.0751 \), \( R^2 = 0.9944 \) depicting the linearity (fig. 1).

Preparation of self-nano emulsifying drug delivery system (SNEDDS)

With the help of Labrafil M 1944 CS (oil), tween 80 (surfactant) and Transcutol P (co-surfactant) in different concentrations, 27 formulations of the SNEDDS based on the solubility (table 2) were prepared and observed visually for their self-emulsifying ability (table 3).

The concentration of extract (150 mg) was kept the same for all the batches. A pseudo-ternary phase diagram was created to identify the region of self-emulsification and concentrations of oil, surfactant and co-surfactant were also optimized for the formulation. The ternary phase diagram of the different batches is shown in fig. 2. Formulation B1 was found clear among all the batches and considered as SNEDDS on the basis of its visual inspection. Batch B10 was found to be transparent and clear but unstable. Creaming was observed in B2, B3, B4, B5, B6, B7, B8, B9, B10, B11, B12, B13, B14, B15, B16, B17, B18, B19, B20, B21, B22, B23, B24, and B25 while phase separation occurred in B1, B3, B4, B5, B6, B7, B8, B9, B11, B12, B13, B14, B15, B16, B17, B18, B19, B20, B21, B22, B23, and B24. It was also observed that 10% of Labrafil M1944 CS is sufficient to solubilize the extract. Fig. 2 showed the ternary phase diagram.

Entrapment efficiency

Entrapment efficiency provides the concentration of the drug entrapped into the carrier. The process was repeated for three times. The average percent entrapment efficiency was found to be 99.63% (n=3), which indicated that most of the added extract was entrapped in SNEDDS.

Particle size and polydispersity index

The particle size and PDI of the optimized batch of SNEDDS were determined by differential light scattering (DLS) technique. The average droplet size and PDI of the optimized batch of SNEDDS were found to be in nano-range i.e. 206.0 nm and 0.156, respectively (fig. 3).
Fig. 2: Ternary phase diagram showing SNEDDS, emulsion and phase

Fig. 3: Particle size and polydispersity index

Fig. 4: Zeta potential of optimized SNEDDS formulation
Zeta potential

The zeta potential of the optimized batch of SNEDDS was found to be -6.84 mV (fig. 4), which was in the acceptable range of -16 to -32 mV. The zeta potential in the range of -16 to -32 mV is appropriate to stabilize the prepared formulation.

TEM analysis

The TEM image clearly showed the droplet size of the optimized formulation and average size was found to be 73.71 nm (fig. 5).

In vitro antioxidant assay

Various concentrations ranging from 0.075-0.75 mg/ml of standard (Ascorbic acid) and formulation were prepared for the activity. The percent inhibition of DPPH by ascorbic acid and SNEDDS was determined (table 4) and IC_{50} values were calculated. The IC_{50} values for the standard and formulation were recorded as 0.53 mg/ml and 0.24 mg/ml, respectively (fig. 6), which clearly showed the improved efficacy of the drug incorporated in SNEDDS.

Table 4: Percent inhibition of standard and SNEDDS observed in in vitro antioxidant activity

| Concentration (mg/ml) | Log concentration (mg/ml) | % inhibition of DPPH (Ascorbic acid) | % inhibition of DPPH (SNEDDS) |
|-----------------------|---------------------------|-------------------------------------|-------------------------------|
| 0.075                 | -1.12494                  | 14.87                               | 31.01                         |
| 0.15                  | -0.82391                  | 32.27                               | 49.05                         |
| 0.3                   | -0.52288                  | 41.77                               | 52.84                         |
| 0.45                  | -0.34679                  | 47.15                               | 58.22                         |
| 0.6                   | -0.22185                  | 50.94                               | 60.75                         |
| 0.75                  | -0.12494                  | 55.37                               | 65.82                         |

In vitro α-amylase inhibition assay

Different concentrations (0.075-0.75 mg/ml) of standard (Acarbose) and formulation were prepared for in vitro study (table 5). The inhibition observed in different concentrations of standard and SNEDDS were recorded and IC_{50} values were calculated (table 5). The IC_{50} values for acarbose and optimized formulation were found to be 0.064 mg/ml and 0.091 mg/ml, respectively (fig. 7). The results revealed that formulation has better anti-diabetic activity than standard.

Table 5: Percent inhibition of standard (Acarbose) and SNEDDS observed in in vitro α-amylase inhibition assay

| Concentration (mg/ml) | Log Concentration (mg/ml) | % Inhibition of α-amylase (Acarbose) | % Inhibition of α-amylase (SNEDDS) |
|-----------------------|---------------------------|-------------------------------------|-------------------------------|
| 0.075                 | -1.12494                  | 58.5                                | 50.14                         |
| 0.15                  | -0.82391                  | 67.76                               | 62.53                         |
| 0.3                   | -0.52288                  | 74.92                               | 90.59                         |
| 0.45                  | -0.34679                  | 86.86                               | 114.02                        |
| 0.6                   | -0.22185                  | 101.34                              | 124.47                        |
| 0.75                  | -0.12494                  | 110.74                              | 139.25                        |
CONCLUSION
The study revealed the potential application of SNEDDS for improving the dispersity, stability, and bioavailability of plant extracts. Ethanolic extract was prepared from dried leaves of *E. japonica* and the average yield of the extract was found to be 11.06%. The SNEDDS formulations were prepared by using Labrafail, tween-80, and Transcutol P as oil, surfactant, and co-surfactant, respectively. The optimized design suggested that 10% of Labrafail M 1944CS, 30% of Tween 80 and 60% of Transcutol P can give SNEDDS with 208 nm mean droplet size, 99.63% drug loading, 0.156 PDI and -6 mV zeta potential. TEM image confirmed the nano-size (less than 100 nm) and the shape of SNEDDS. In vitro antioxidant and antidiabetic activities of SNEDDS were observed to be comparable with ascorbic acid and acarbose, respectively, which revealed the potential of SNEDDS formulation in diabetes management.

AUTHORS CONTRIBUTIONS
The authors declare that this work was performed by all authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by all of them. All authors approved and read the manuscript for publication.

CONFLICT OF INTERESTS
The authors have declared no conflict of interest

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