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Development and In Vitro Characterization of Nanoemulsion Containing *Hippophaes rhamnoides* Aqueous Extract

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ABSTRACT

Objective: *Hippophaes rhamnoides* is a member of the Elaeagnaceae family and grows in many countries of the world such as Turkey, France, Germany, Norway, and Russia. *Hippophaes rhamnoides* extracts have antioxidant, anticancer, antidiabetic, antiviral, and cardiovascular protective effects. Nanoemulsions are suitable systems for formulations of herbal extracts. The aim of this study was to develop and *in vitro* characterize nanoemulsion formulation containing *Hippophaes rhamnoides* aqueous extract.

Methods: *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion or blank nanoemulsion formulations were prepared using ethyl oleate, lipoid S100, Kolliphor RH40, Pluronic F68, Dimethyl sulfoxide (DMSO), and ultrapure water. The droplet size, polydispersity index, and zeta potential values of nanoemulsions were determined, and pH measurement, Fourier-transform infrared spectroscopy (FT-IR), and rheological analyses were also performed.

Results: The droplet size and polydispersity index values of nanoemulsions were found to be <200 nm and <0.3, respectively, which indicates monodispersity. The zeta potential values of blank nanoemulsion and *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion were determined as (–)28.51 ± 2.61 and (–)30.11 ± 2.02 mV, respectively. FT-IR results showed that the extract is completely dissolved in the oil phase of formulations.

Conclusion: The nanoemulsion formulation containing *Hippophaes rhamnoides* aqueous extract was successfully prepared and characterized. The formulation may be beneficial for the usage of *Hippophaes rhamnoides* aqueous extract.

Keywords: Hippophaes rhamnoides, in vitro characterization, nanoemulsion

INTRODUCTION

Emulsion is defined as heterogeneous systems in which 2 immiscible liquids are dispersed as droplets within each other.[1] Nanoemulsion (NE), which is a subtype of emulsion systems, has a droplet size below 1000 nm and can be prepared as oil in water or water in oil. Nanoemulsions generally have droplet size in the range of 100-500 nm.[2,3] They are kinetically stable systems and can be prepared by high-energy methods (such as high-pressure homogenization, ultrasonication, and microfluidization) and low-energy methods (such as phase inversion and self-emulsification methods).[2] They can be administered via different routes (e.g., oral, topical, and parenteral). Nanoemulsions are preferred systems because they increase the solubility and bioavailability of active compounds and provide reproducible bioavailability.[4,5] Nanoemulsions can be prepared as dosage forms such as spray, cream, gel, foam, and aerosol.[3]

Hippophaes rhamnoides is a member of the Elaeagnaceae family and grows in many countries of world such as Turkey, France, Germany, Norway, and Russia. It is a valuable plant and has gained attention worldwide because of its medicinal and nutritional properties.[6] Moreover, it has been reported to have antioxidant activity, cardioprotective activity, antitumor effect, antidiabetic effect, wound healing properties, and antimicrobial and antiviral activities.[7–12] Bioactive compounds of *H. rhamnoides* are flavonoids, phenols, carotenoids, vitamins, fatty acids, terpenes, and sterols.[13,14]

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METHODS

Materials

In this study, ethyl oleate (Merck, Germany), lipoid S100 (Lipoid, Germany), Kolliphor RH40 (Sigma, Germany), Pluronic F68 (BASF, Germany), DMSO (Lab-Scan, Ireland) were used. The water was purified by Direct-Q®3 UV water purification system (Millipore, USA).

Preparation of Hippophaes rhamnoides Aqueous Extract

First, the aerial parts of *H. rhamnoides* were pulverized using a laboratory blender. About 250 mL of ultrapure water was added to 10 g powder and kept in a horizontally shaking water bath for 48 hours at 50°C. It was filtered every 24 hours, and fresh ultrapure water was added to enable the removal of plant material. After 48 hours, the filtrates were collected and evaporated to almost dryness (50°C, 60 rpm). The concentrated filtrate was frozen at −20°C and then lyophilized at −55°C for 24 hours. The dry *Hippophaes rhamnoides* aqueous extract (AE-Hr) was stored in airtight containers in a refrigerator (2-8°C), protected from light, for further studies.

Preparation of Nanoemulsion Formulations

Hippophaes rhamnoides aqueous extract-containing nanoemulsion (AE-Hr-NE) and blank nanoemulsion (B-NE) formulations were prepared using a high-energy method. First, the oil [ethyl oleate (5%) and lipoid S100 (1%)] and water [Kolliphor RH40 (1%), Pluronic F68 (1%), and ultrapure water] were prepared separately. The extract (10 mg) was added to the oil phase after dissolving in DMSO. Under magnetic stirring (750 rpm), the water phase was added to the oil phase, and coarse emulsion was prepared. The coarse emulsion was first homogenized with a high-speed mixer (Ultraturrax T-10; 25000 rpm, 5 minutes), and then ultrasonication (40% power, 15 minutes) was performed to ensure nanosized droplets. Blank nanoemulsions were prepared by the same procedure but without the addition of the extract.

Formulation Characterization

Droplet Size, Polydispersity Index, Zeta Potential, and Morphological Analysis

Zetasizer Nano ZSP (Malvern Ins., Ltd, UK) was used to determine the mean droplet size, polydispersity index (PDI), and zeta potential values of the NE formulations. In addition, the AE-Hr-NE was imaged using transmission electron microscopy (TEM) (Hitachi HighTech HT7700, Japan). After dilution for 100 times, the AE-Hr-NE was placed on a copper grid and dried at room temperature over 24 hours. Images of the grids were then obtained at 120 kV.

pH

The pH values of NEs were determined at room temperature using a pH meter (Thermo Scientific, Orion 3 Star, USA).

Rheology

A Brookfield RV DV2T cone and plate viscometer were used to measure the viscosity of the NEs at room temperature.

FT-IR Analysis

FT-IR analyses (4000-400 cm^{-1}) of the extract and NEs were performed using Fourier transform infrared spectroscopy (Shimadzu IRSprit-T).

Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences Version 22.0 (IBM SPSS Corp., Armonk, NY, USA) software. The "independent *t*-test" was used to compare the differences between 2 independent samples. The significance of the difference between test results was determined, and the difference was accepted to be significant if $P < .05$.

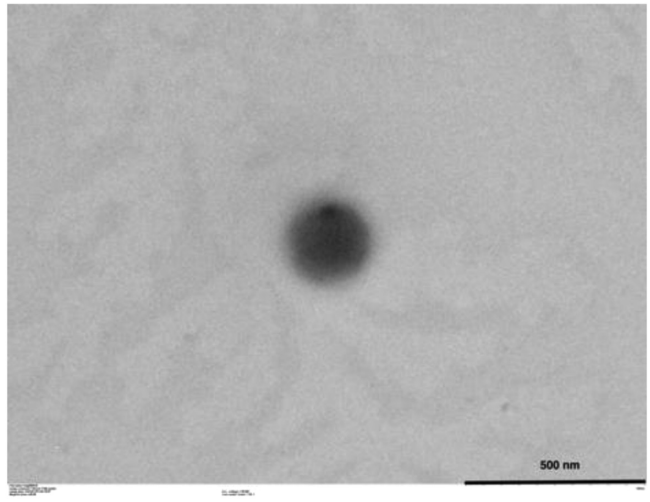

Figure 1. The transmission electron microscopy image of *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion.

RESULTS

Droplet Size, Polydispersity Index, Zeta Potential, and Morphological Analysis

The droplet size, PDI, and zeta potential values of the prepared NE formulations are given in Table 1. The TEM image of AE-Hr-NE is shown in Figure 1.

pH Determination

The pH values of the NE formulations are given in Table 2.

FT-IR Analysis

FT-IR spectra of AE-Hr and the prepared NE formulations are shown in Figure 2.

Rheological Analysis

Rheograms of the prepared NE formulations are given in Figure 3.

DISCUSSION

Droplet Size, Polydispersity Index, Zeta Potential, and Morphological Analysis

Droplet size and droplet size distribution are parameters that affect bioavailability and physical and chemical stability and are very important for the characterization of NE formulations.[15–18] The average droplet sizes and PDI values of the prepared NE formulations are given in Table 1. When evaluated statistically, it was

Table 1. The Mean Droplet Size, PDI, and Zeta Potential Values of NEs ($X \pm SD$, n = 9)

Formulation	Droplet Size (nm)	PDI	Zeta Potential (mV)
B-NE	176.89 ± 4.79	0.257 ± 0.019	−28.51 ± 2.61
E-NE	183.07 ± 9.53	0.295 ± 0.045	−30.11 ± 2.02

B-NE, blank nanoemulsion; E-NE, extract-containing nanoemulsion; NE, nanoemulsion; PDI, polydispersity index; *X*, mean.

Table 2. pH Values of NE Formulations ($X \pm SD$; n = 9)

Formulation	pH
B-NE	5.19 ± 0.06
AE-Hr-NE	5.15 ± 0.01

AE-Hr-NE, *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion; B-NE, blank nanoemulsion; *X*, mean.

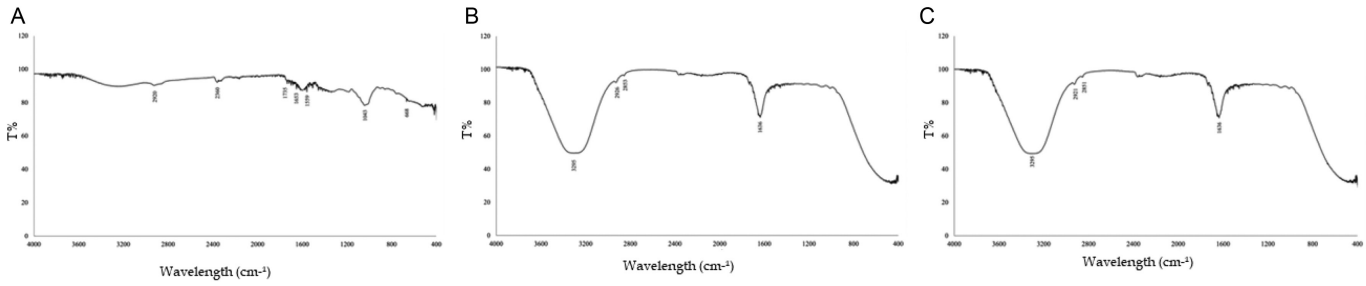

Figure 2. FT-IR spectra of *Hippophaes rhamnoides* aqueous extract (A), blank nanoemulsion (B), and *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion (C).

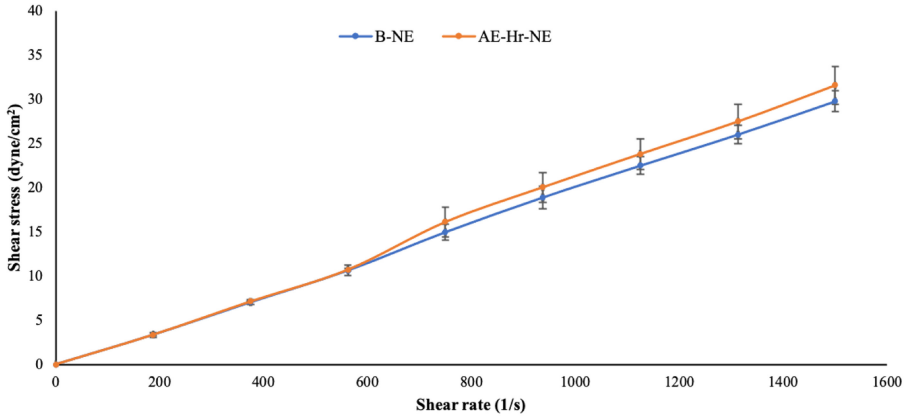

Figure 3. Flow rheograms of blank nanoemulsion and *Hippophaes rhamnoides* aqueous extract-containing nanoemulsion formulations ($X \pm SD$; n = 3).

determined that the increase in droplet size observed with the addition of the extract was not significant ($P > .05$). The PDI value expresses the uniformity of the droplet size of the formulation. In our study, it was observed that the PDI values of the NEs were <0.3 and the droplet size distribution was found to be in a narrow range. A PDI value <0.3 indicates good homogeneity.[19,20] Zeta potential is a term that describes the electrokinetic potentials of droplets and is very important in the evaluation of physical stability of colloidal dispersions. As a general rule, zeta potential values above ±30 mV are indicative of good stability. However, zeta potential values above ±20 mV can also provide sufficient stabilization in cases where high-molecular-weight surfactants and steric stabilization are also concerned. Non-ionic surfactants contribute to stability by forming a steric barrier between droplets. [21,22]

The TEM image of AE-Hr-NE is shown in Figure 1. It was observed that the droplets were nanosized and very close to spherical shape.

pH Determination

The presence of ions or the change in pH in an NE formulation is of great importance in formulation design and subsequent stability studies.[23] The pH values of the NEs are given in Table 2. There was no change in the pH value with the addition of extract to the formulations ($P > .05$).

FT-IR Analysis

In our study, FT-IR analysis was performed in order to obtain information about the functional groups in the structure of the formulation components and to determine whether there is an interaction between the components of the formulations and the extract. When the FT-IR spectra of B-NE and AE-Hr-NE are examined, it is seen that both spectra are quite similar, and the characteristic peaks of the extract are not in the spectrum of AE-Hr-NE (Figure 2). This indicates that the extract is dispersed at the molecular level in the NE formulation.[24]

Rheological Analysis

The relationship between shear rate and shear stress was determined in order to evaluate the flow properties of the prepared NEs (Figure 3). The viscosity values for B-NE and AE-Hr-NE were found to be 1.98 ± 0.08 and 2.10 ± 0.14, respectively, at a shear rate of 1500 s^{-1} at room temperature.

Ethics Committee Approval: Ethics committee approval was not required as no in vivo studies were included in this study.

Informed Consent: Since there was no in vivo study in this study, no patient consent was required.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.F.K., A.B.U.K., M.C.; Design – Y.F.K., A.B.U.K., M.C.; Supervision – A.B.U.K., M.C.; Resources – A.B.U.K., M.C.; Materials – A.B.U.K., M.C.; Data Collection and/or Processing – Y.F.K., S.C.; Analysis and/or Interpretation – S.C., Y.F.K., A.B.U.K., M.C.; Literature Search – S.C., Y.F.K., A.B.U.K., M.C.; Writing Manuscript – Y.F.K., A.B.U.K., M.C.; Critical Review – A.B.U.K., M.C.

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