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Research Article

Development and in Vitro Characterization of Nanoemulsion and Nanoemulsion Based Gel Containing Artemisia Dracunculus Ethanol Extract

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ABSTRACT:

Tarragon, or Artemisia dracunculus, is a member of the Asteraceae family that grows wild in Eastern Anatolia of Turkey. According to previously published studies, Artemisia dracunculus extracts possess antibacterial, antifungal, antioxidant and anti-inflammatory effects. Thus, these extracts can be used to heal wounds. Nanoemulsion (NE) is a suitable dosage form for the application of active substances/compounds via the skin. The aim of this study is to develop and in vitro characterize NE and NE-based gel (NEG) formulations containing Artemisia dracunculus ethanol extract.

Methods: Extract-containing (E-NE) or blank (B-NE) NE formulations were prepared using ethyl oleate, Lipoid S100, Tween 80, Pluronic F127, DMSO, and ultrapure water. NaCMC was added to NE formulations to obtain NE-based gels. The droplet size, PDI and zeta potential values of NEs were determined; pH measurement, FT-IR and rheological analyzes were also performed for NEs and NEGs.

Results: The droplet size and zeta potential values of B-NE and E-NE were found as 139.13±5.15 nm and 135.59±4.81 nm, (-)27.53±2.05 mV and (-)26.28±3.21 mV, respectively. Also, PDI values of NE formulations were <0.3, indicating monodispersity. The pH values of NEs and NEGs were found in the range of 4.26±0.10 - 6.16±0.03, using suitably for topical application. In addition, NEGs formulations showed a pseudoplastic behavior which is important for the topical application. FT-IR results showed that the extract is completely dissolved in the oil phase of formulations.

Conclusion: The NE and NEG may be useful for the topical application of Artemisia dracunculus ethanol extract.

Keywords: Artemisia dracunculus, extract, nanoemulsion, nanoemulsion-based gel, topical application.

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1. INTRODUCTION

Tarragon, or *Artemisia dracunculus*, is a member of the Asteraceae family that grows wild in Eastern Anatolia of Turkey [1]. *Artemisia dracunculus*, which has its origins in Siberia and Mongolia, can also be found growing naturally throughout Central Asia, Eastern Europe, and the Mediterranean. It has been used for its analgesic, hypnotic, antiepileptic, antipyretic, and anti-inflammatory effects in traditional Asian medicine (especially in Iran, Azerbaijan, India and Pakistan). Besides, Artemisia dracunculus is commonly used to treat skin wounds, allergic rashes, dermatitis, and irritations in Central Asia and Russia.

Some articles emphasized that, *Artemisia dracunculus* extracts can be used to heal wounds due to their antibacterial, antifungal, antioxidant, and anti-inflammatory effects [2,3].

Minda et al. [4] investigated the wound healing activities of some *Artemisia sp.* (*Artemisia annua , Artemisia dracunculus, Artemisia absinthium*). For this purpose, the authors first prepared their ethanol extracts and later showed that these extracts had high antioxidant activity comparable to ascorbic acid by DPPH test. In addition, the wound healing effects of the three ethanol extracts (at 100 µg/mL concentrations) were evaluated on human keratinocyte cells. The obtained results showed that stimulated keratinocyte proliferation and wound closure achieved. On the other hand, in ovo evaluation has shown that these extracts were well tolerated and have anti-irritation properties. The authors stated that ethanol extracts of *Artemisia sp.*, which are rich in polyphenolic compounds, can be a low-cost and safe alternative in wound treatment [4].

NEs are referred to in the literature as mini-emulsions, ultrafine emulsions, submicron emulsions. They are colloidal dispersions of two immiscible phases (water phase and oil phase, in combination with suitable surfactant/s. The droplet sizes of NEs are generally in the range of 100-600 nm, and NEs are kinetically stable systems. In addition, NEs are efficient drug delivery systems for increasing the solubilization of poorly water-soluble drugs. They can be administered via different routes (parenteral, topical, oral, etc.). For topical application, NEs are considered efficient systems that favor drug penetration into skin layers. Their small-sized droplets with high surface area enable distribution homogeneously on the skin [5- 7]. They have a high potential in the treatment of skin diseases and wound healing due to these properties [8].

The penetration of the active compound (essential oils, other lipophilic compounds, etc.) to the deep layers of the skin is significant in the emergence of the expected effect after topical application. Therefore, with NE formulations used, the effectiveness of these active compounds with wound healing properties can be increased due to their increased penetration [8-10]. However, due to its low viscosity, the poor retention on skin and spreadability issue limit the clinical use of NE formulations for topical application. Therefore, nanoemulgel, basically an oil-in-water nanoemulsion-based topical gel formulation, has been proposed as a strategy to overcome this problem [11,12].

The gelling system, which enhances the viscosity of NEs, is prepared using gel-forming agents such as Carbopol, chitosan, hydroxyl propyl methyl cellulose [4, 11-13]. The aim of this study is to develop and in vitro characterize NE and NEG formulations containing Artemisia dracunculus ethanol extract for topical application.

2. MATERIALS AND METHODS

2. 1. Materials

In this study, ethyl oleate (Merck, Germany), Lipoid S100 (Lipoid, Germany), Tween 80 (Merck, Germany), Pluronic F127 (BASF, Germany), DMSO (Lab-Scan, Ireland) were used. Also, the water was purified by Direct-Q®3 UV water purification system (Millipore, USA).

2.2. Methods

2.2.1. Ethanol Extract Preparation

10 g of the powder of dried leaves of *Artemisia dracunculus* suspended in ethanol (500 mL) was kept in horizontally shaking water bath at 50°C for 72 hours. This mixture was filtered every 24 hours, and extraction was continued by adding ethanol. The filtrates obtained were combined and the organic solvent was evaporated under reduced pressure at 50°C. The extract was stored for further studies in a refrigerator (2-8 °C) in an airtight bottle and protected from light.

2.2.2. Artemisia dracunculus Ethanol Extract-Containing Formulations Preparation

A high energy method was used for the preparation of B-NE and E-NE formulations. Briefly, oil (ethyl oleate and Lipoid S100) and water (Tween 80, Pluronic F127 and ultra-pure water) phases were prepared separately. The extract was dissolved in DMSO and then added to the oil phase. The aqueous phase was added to the oil phase under magnetic stirring to obtainthe coarse emulsion. Later, this emulsion was homogenized at 25000 rpm for 5 min using a T10 Ultraturrax (IKA, Germany), and then ultrasonicated for 15 min (40% power; Sonoplus HD 2070; Bandelin Electronics, Germany) to ensure nano-sized droplets. B-NEs were prepared according to the above procedure without the extract. NaCMC (1%) was added to NEs and stirred overnight at room temperature on a magnetic stirrer to obtain NE-based gels (NEGs).

2.2.3. Formulations Characterization

2.2.3.1. Droplet Size, Polydispersity Index (PDI), 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 E-NE was imaged using TEM (Hitachi HighTech HT7700, Japan). After dilution 100 times, the E-NE was placed on a copper grid and dried at room temperature over 24 h. Images of the grids were then obtained at 120 kV.

2.2.3.2. pH

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

2.2.3.3. Rheology

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

2.2.3.4. FT-IR Analysis

FT-IR analyzes (4000-400 cm-1) of the extract, NEs, and NEGs were performed using Fourier transform infrared spectroscopy (Shimadzu IRSprit-T).

2.2.3.5. Statistical analyses

Statistical analyses were performed using SPSS Statistics Version 22.0 (SPSS Inc., Chicago, USA) software. The "Independent t- test" was used to compare the differences between two independent samples. The significance of the difference between test results was determined and the difference was accepted to be significant if $p < 0.05$.

3. RESULTS & DISCUSSION

3.1. Droplet Size, PDI, Zeta Potential and Morphological Analysis

The droplet size, PDI and zeta potential values of the NE formulations are given in Table 1. Statistical comparison of droplet sizes of B-NE and E-NE showed that the droplet size did not change with the addition of the extract to the formulation ($p>0.05$). Zeta potential value, which is a significant parameter for the physical stability of colloidal dispersions, is considered sufficient for stability if it is ±20 mV and above in the presence of both electrostatic and steric barriers [14]. There was no significant difference between the zeta potential values of B-NEs and E-NEs ($p>0.05$). Furthermore, PDI values of NEs were less than 0.3, indicating monodispersity (15).

Table 1. The mean droplet size, PDI and zeta potential values of NEs.

Formulation	Droplet size (nm)	PDI	Zeta Potential (mV)
B-NE	139.13±5.15	0.214±0.023	-27.53±2.05
E-NE	135.59±4.81	0.238±0.022	-26.28±3.21

The TEM image of E-NE is shown in Figure 1. Figure 1 shows that the droplets of NE were approximately spherical.

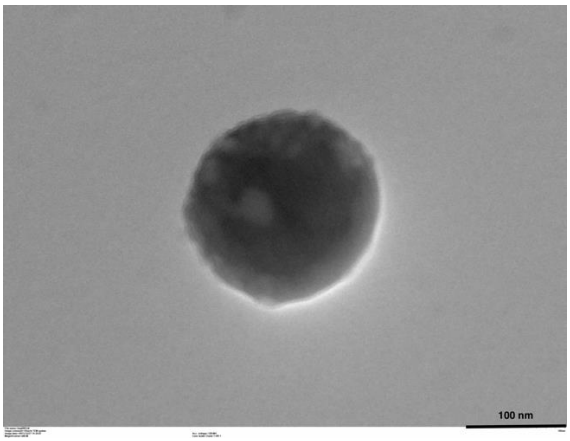

Figure 1. The TEM image of E-NE.

3.2. pH

It was determined that the mean pH values of the B-NE and E-NE formulations were 5.52±0.06 and 4.26±0.10, respectively, and the pH value of the NE formulation decreased in the presence of the extract ($p<0.05$). In addition, pH values of B-NEG and E-NEG formulations were 6.16±0.03 and 5.84±0.07, respectively, and an increase in pH

values after gelation was observed ($p<0.05$). Formulations with very high or very low pH values cause irritation when applied to the skin. Human skin pH is generally acidic, but the pH ranges widely from 4.0 to 7.0 [15, 16]. The pH values of the NE and NEG formulations prepared in our study are suitable for application to the skin without causing irritation.

3.3. Rheology

For topical formulation, another important parameter is Viscosity related to the applicability of the formulations. The viscosity values of NEs and NEGs are shown in Figure 3. Flow behaviors of NEs and NEGs were described using "power-law the flow behavior index (n) was calculated. While n is 1 for Newtonian systems, it is <1 for shear-thinning systems. In our study, the n values of the NE formulations were very close to 1 (0.9997 for B-NE and 0.9998 for E-NE), and these formulations exhibited Newtonian flow. On the other hand, the n values of the NEG formulations were less than one (0.7088 for B-NEG and 0.7099 for E-NEG), and they showed pseudoplastic flow. The shear-thinning flow is significant for NEG formulations applied to the skin because a thick product thins under shear stress and can spread smoothly over the skin [5,17].

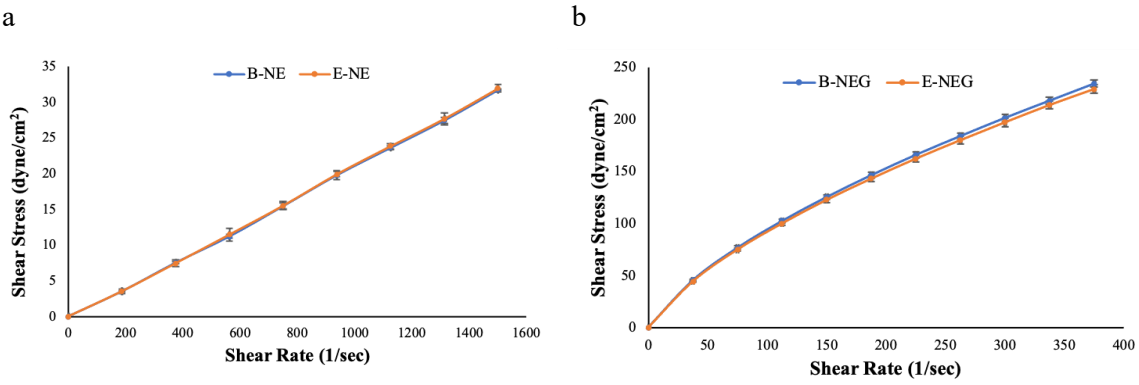

Figure 2. Flow curves of NEs (a) and NEGs (b).

3.4. FT-IR Analysis

FT-IR spectra of the extract, NE, and NEG formulations were taken to determine the structural properties of the extract and the interactions between the extract and other formulation components. The FT-IR spectra of blank formulations (B-NE or B-NEG) and extract-containing formulations (E-NE or E-NEG) were similar (Figure 3). The characteristic peaks of the extract were not observed in the FT-IR spectra of E-NE and E-NEG (Figure 3). Thus, it was confirmed that the extract is dispersed in the formulations at the molecular level [5, 18].

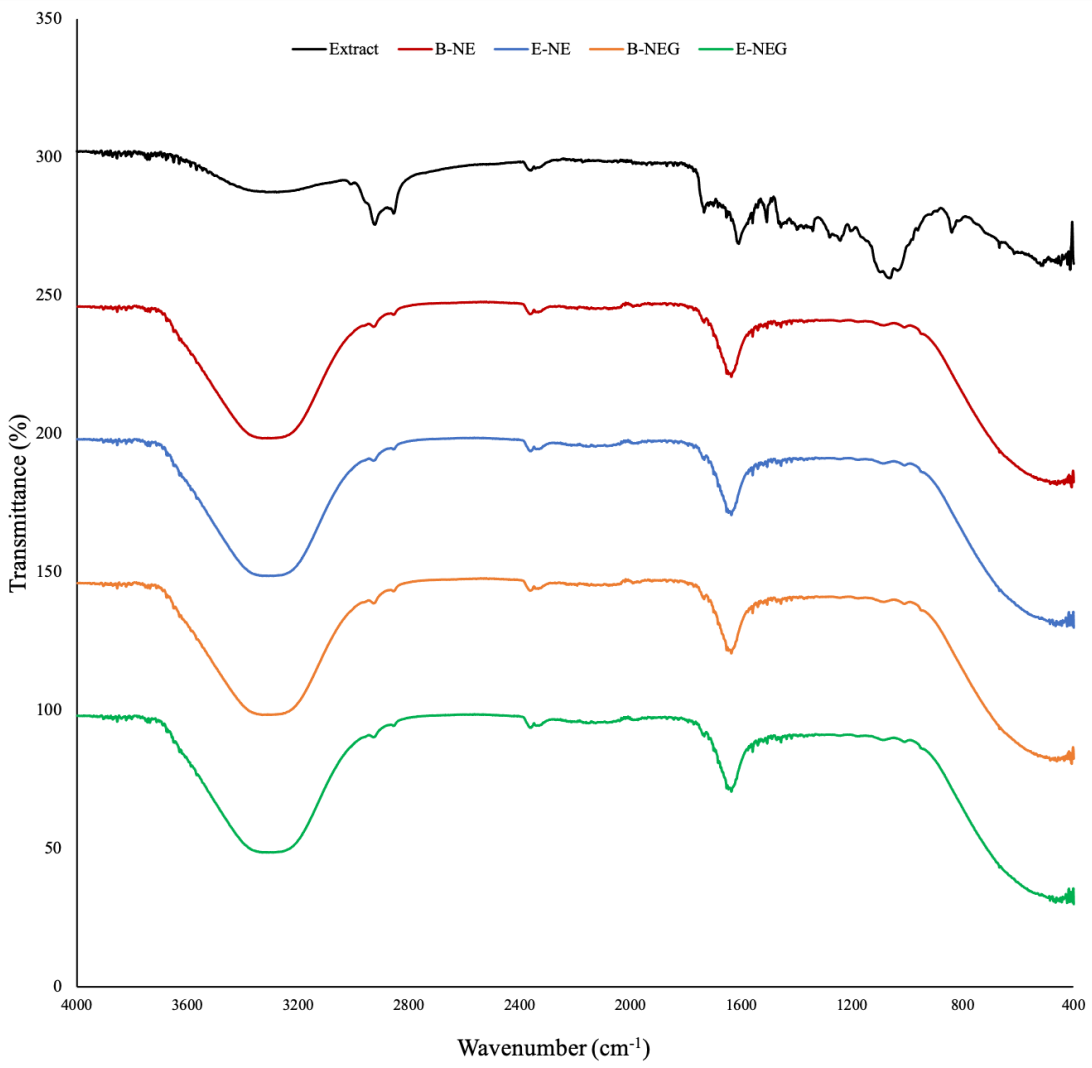

Figure 3. FT-IR spectra of the extract, NEs and NEGs.

4. CONCLUSION

In this study, NE and NEG formulations were prepared successfully and in vitro characterized. The NE and NEG may be useful for the topical application of *Artemisia dracunculus* ethanol extract.

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Conflict of Interest

Author has no personal financial or non-financial interests.

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