EURASIAN JOURNAL OF ACADEMIC RESEARCH
Innovative Academy Research Support Center
IF = 9.3 www.in-academy.uz/index.php/ejar

IN VITRO ANTIOXIDANT ACTIVITY EVALUATION OF SNEDDS-BASED FORMULATIONS USING DPPH ASSAY

Savurova M.A.
Parmonova M.F.
Tashkent Scientific Research Institute of Vaccines and Serums, Republic of Uzbekistan
Email: tashrivs@umail.uz
https://doi.org/10.5281/zenodo.19565199

ARTICLE INFO

Received: 06th April 2026
Accepted: 13th April 2026
Online: 14th April 2026

KEYWORDS

SNEDDS; antioxidant activity; DPPH assay; plant extracts; curcumin; grape seed extract; Melissa officinalis; controlled release; nanoemulsion; bioavailability enhancement; free radical scavenging.

ABSTRACT

This study investigates the in vitro antioxidant activity of plant-derived extracts and self-nanoemulsifying drug delivery systems (SNEDDS)-based formulations using the DPPH assay. Oxidative stress is a key factor in the pathogenesis of various diseases, which has led to increased interest in antioxidant-rich formulations. However, many plant-derived bioactive compounds suffer from poor solubility and limited bioavailability, restricting their therapeutic potential.

To address this limitation, SNEDDS formulations were developed to enhance solubility and improve the functional performance of hydrophobic antioxidants. Antioxidant activity was evaluated using the DPPH radical scavenging method, with absorbance measured at 517 nm. The percentage of inhibition was calculated to assess the free radical scavenging capacity of the samples.

The results demonstrated that antioxidant activity varied depending on the type of extract and formulation. Reference standards (ascorbic acid and DPPH control) showed the highest activity (~95–100%), confirming the validity of the method. Among the tested samples, curcumin, grape seed, and Artemisia annua-based formulations exhibited significant antioxidant activity, with a clear time-dependent increase, indicating controlled release behavior. Melissa officinalis extract demonstrated the highest overall activity among plant-based samples. In contrast, formulations containing gold nanoparticles showed negligible antioxidant effects.

The findings confirm that SNEDDS-based systems enhance the antioxidant performance of plant-derived compounds and enable sustained release profiles. This approach represents a promising strategy for improving the delivery and therapeutic efficacy of antioxidants in pharmaceutical and dermatological applications.

Introduction. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is involved in the development of various pathological conditions, including inflammation, cancer, and neurodegenerative diseases. Therefore, there is growing interest in developing formulations with strong antioxidant activity.

Plant-derived bioactive compounds, particularly polyphenols and flavonoids, are well known for their free radical scavenging properties. Extracts such as *Melissa officinalis*, *Calendula officinalis*, grape seed, and curcumin have been widely studied for their antioxidant potential (Rice-Evans et al., 1997). However, their application is often limited by poor solubility and low bioavailability.

Self-nanoemulsifying drug delivery systems (SNEDDS) have emerged as an effective approach to improve solubility and enhance the functional activity of hydrophobic compounds (Shakeel et al., 2008). The DPPH assay is a widely used method for evaluating antioxidant activity based on the reduction of a stable free radical, measured at 517 nm (Brand-Williams et al., 1995).

The aim of this study was to evaluate the in vitro antioxidant activity of plant extracts and SNEDDS-based formulations using the DPPH assay, with emphasis on formulation effects and release behavior.

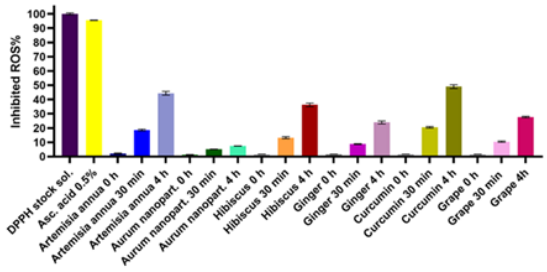
Materials and Methods. A freshly prepared DPPH solution (0.06 mM) was used for all experiments by dissolving 2.365 mg of DPPH in 100 mL of 96% ethanol. The solution was protected from light to prevent degradation. Test samples, including plant extracts and SNEDDS-based formulations, were diluted in phosphate-buffered saline (PBS), and 1 mL of each sample was used for analysis.

For the DPPH assay, 1 mL of the sample solution was mixed with 2 mL of DPPH reagent. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes to allow interaction between antioxidants and DPPH radicals. A control sample (A_0), containing DPPH solution without antioxidant, was prepared, while ethanol served as the blank.

Following incubation, absorbance (A_s) was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of radical scavenging activity (I%) was calculated according to the following equation:

I%=A0−AsA0×100I\% = \frac{A_0 - A_s}{A_0} \times 100I%=A0A0−As×100

where A_0 represents the absorbance of the control and A_s represents the absorbance of the sample.



Results and Discussion. The DPPH assay showed that the reference standards (DPPH and ascorbic acid 0.5%) exhibited the highest antioxidant activity (~95–100%), confirming the reliability of the method.

Among the samples, antioxidant activity varied depending on extract type and incubation time. *Artemisia annua*, hibiscus, and curcumin formulations

showed clear time-dependent increases in activity, indicating gradual release of active compounds. Curcumin and grape seed extracts demonstrated the highest activity at 4 hours (~50%), consistent with their known polyphenol content. In contrast, gold nanoparticle-containing samples showed minimal activity (<5%), suggesting negligible radical scavenging effect. Ginger extract exhibited moderate activity with slight time-dependent improvement.

Overall, the results highlight that antioxidant activity depends on both formulation and release behavior, with several systems showing enhanced effects over time.

Conclusion. The present study demonstrated that SNEDDS-based formulations containing plant-derived bioactive compounds exhibit significant antioxidant activity as evaluated by the DPPH assay. The antioxidant capacity varied depending on the type of extract, with *Melissa officinalis* showing the highest activity.

The observed time-dependent increase in antioxidant activity confirms the controlled release behavior of SNEDDS systems, which may enhance the therapeutic potential of incorporated bioactive compounds. These findings suggest that SNEDDS represent a promising approach for improving the delivery and efficacy of antioxidant agents in pharmaceutical and dermatological applications.

References:

1. Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25–30.
2. Rice-Evans, C., Miller, N.J. & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2, 152–159.
3. Shakeel, F. et al. (2008). Self-nanoemulsifying drug delivery system: a review. *AAPS PharmSciTech*, 9, 1236–1243.