# Sonication of smoke tree extract-loaded liposomes: the antioxidant potential of particles

ALEKSANDRA JOVANOVIĆ[1*], MILENA MILOŠEVIĆ[2], MIROSLAV NOVAKOVIĆ[2] AND ALEKSANDAR MARINKOVIĆ[3]

[1]*Institute for the Application of Nuclear Energy INEP, University of Belgrade, Banatska 31b, 11080 Belgrade, Serbia*
[2]*Institute of Chemistry, Technology, and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia*
[3]*Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia*
**Corresponding author: ajovanovic@inep.co.rs*

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**With the aim to examine the radical scavenging activity of smoke tree extract-loaded liposomal particles before and after ultrasound exposure (45 or 70% amplitude and 15 or 30 min), ABTS and DPPH tests were employed. The antioxidant activity of the pure extract was 11.37 ± 0.52 μmol Trolox equivalent (TE)/mL and 79.7 ± 0.5%. Multilamellar liposomes with extract showed significantly higher antioxidant activity in both assays (12.02 ± 0.54 μmol TE/mL and 81.9 ± 0.4%) compared to sonicated liposomes (10.75-11.00 μmol TE/mL and 79.3-80.9%) and pure extract. There was no significant difference between the ABTS radical scavenging activity of the liposomes treated by different amplitudes and times, while prolonged sonication and a higher amplitude caused a significant drop in the anti-DPPH capacity of extract-loaded liposomal vesicles. The presented results and the differences between the obtained data provide a good insight into the overall antioxidant capacity of smoke tree extract-loaded multilamellar and sonicated unilamellar liposomal vesicles.**

**Keywords:** antioxidants; liposomes; smoke tree; sonication

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## 1. INTRODUCTION

Smoke tree (*Cotinus coggygria* Scop.) from the family Anacardiaceae is an ornamental tree or large bush that has medicinal properties and multiple biological activities (Matić et al., 2011, 2016; Teixeira Da Silva et al., 2018). The plant has a wide distribution, including southern Europe, the Mediterranean, Moldova, the Caucasus, the Himalayas, and central China (Matić et al., 2016). The species is an important source of essential oil and extracts with a wide range of health-promoting effects (Matić et al., 2016). Various properties, including antioxidant, antibacterial, antifungal, antiviral, anticancer, antigenotoxic, hepatoprotective, and anti-inflammatory have been demonstrated for all parts of the plant by in vivo and in vitro studies (Matić et al., 2011, 2016; Teixeira Da Silva et al., 2018). The antioxidant potential of smoke tree extracts and essential oil is a biological property of great interest because the mentioned formulations can preserve food, pharmaceutical, and cosmetic products from the toxic and degrading effects of oxidants and/or free radicals (Maestri et al., 2006). In traditional medicine, its syrup showed the potential to protect the liver from chemical damage, reduce the tension of the choledochal sphincter, enhance bile flow, and raise immunity (Shen et al., 1991). Ethanol infusions of the wooden parts of the smoke tree were used in the treatment of gastric ulcers, diarrhea, cancer, and eye ailments, and as a cholagogue and antipyretic agent (Matić et al., 2016).

The encapsulation of the extract in various carriers, such as liposomes, can provide a longer and controlled release of its bioactives, i.e., antioxidants, as well as their protection. Additionally, disruption of multilamellar liposomal vesicles (MLVs) using sonic energy (sonication) by ultrasound bath or probe can provide small unilamellar vesicles (SUVs) with improved characteristics (Rieth and Lozano, 2020). Therefore, in the present study, the antioxidant property of smoke tree extract-loaded liposomes (MLVs and SUVs) was examined.

## 2. MATERIALS AND METHODS

### 2.1. Extract preparation

Smoke tree (collected in Belgrade, Serbia) extract was prepared using 5 g of the wooden part (dried material was grinded in the laboratory mill) and 200 mL of 80% ethanol (Fisher Science, United Kingdom) in an ultrasound bath (Sonorex Super RK, Bandelin, Germany) for 30 min. Erlenmeyer flask (250 mL) was covered with aluminum foil to avoid light exposure and ethanol evaporation. The obtained extract was filtered through a cellulose filter (fine pore, 0.45 μm). The extract was stored at 4 °C in a dark place until further experiments.

### 2.2. Liposomal preparation and sonication

Smoke tree extract-loaded MLVs were prepared using a previously published proliposome procedure (Jovanović et al., 2022). Ethanol extract (20 mL) was mixed with 2 g of phospholipids (soy L-α-phosphatidylcholine, Avanti Polar Lipids, USA), and heated to 60 °C for 30 min. After cooling, ultra-pure water (Simplicity UV® water purification system, Merck Millipore, Germany) was added in small portions to a total volume of 20 mL, and the dispersion was stirred for 2 h at 800 rpm. Plain liposomes (without active compounds) were prepared as a control.

With the aim to produce SUVs, an ultrasound probe, Sonopuls (Bandelin, Berlin, Germany), at 45% amplitude for 15 min (40 s on-10 s off) or at 70% amplitude for 30 min (40 s on-10 s off) was employed. The sample temperature was 25 °C; a flask with the liposomes was continuously cooled using ice coating during the sonication and the temperature was measured and controlled.

### 2.3. Determination of size and zeta potential of liposomes

The obtained liposomes' particle size and zeta potential were measured in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., Malvern, UK). Each sample was diluted 200 times and measured three times at 25 °C. The measurement was repeated after three months for the extract-loaded liposomes.

### 2.4. Determination of antioxidant potential (ABTS and DPPH assays)

The ABTS and DPPH radical scavenging capacity of pure smoke tree extract and obtained MLVs and SUVs with extract were examined using spectrophotometric methods. The absorbance was measured using the UV Spectrophotometer UV-1800, Shimadzu, Japan. The measurements were performed on the 1st day and after three months of storage at 4 °C. In the ABTS assay, $ABTS^{\bullet+}$ solution (2 mL) was mixed with liposomes or extract (20 μL of the solution diluted with water in a ratio 1:10) (Li et al., 2013). After 6 min of incubation in the dark, the absorbance was measured at 734 nm. The results are expressed as μmol Trolox equivalent (TE)/mL. In the DPPH assay, non-diluted liposomes or extract (20 μL) were mixed with 1.8 mL of ethanol $DPPH^{\bullet}$ radical solution (Xi and Yan, 2017). After 20 min of incubation in the dark, the absorbance was measured at 517 nm. The results are expressed as the percentage of neutralization of free DPPH radicals. All reagents used in the antioxidant assays were from Sigma Aldrich (Germany).

## 3. RESULTS AND DISCUSSION

In the present study, smoke tree ethanol extract was encapsulated in liposomal particles that were further exposed to sonication. The antioxidant potential of prepared liposomal systems with extract (MLVs and two types of SUVs) was examined using two antioxidant assays. The particle size of MLVs with extract was 3131 ± 17 nm, while the size of SUVs with extract was from 272.9 to 512.6 nm. The size of empty MLVs was 2125 ± 48 nm, whereas the vesicle size of empty SUVs was from 141.7 to 217.9 nm. The zeta potential of the extract-loaded liposomes amounted to –27.7 ± 0.5 mV (for MLVs) and ~ –12.8 mV (for SUVs), while the mentioned parameter was significantly lower for empty parallels (< 10 mV). The mentioned parameters did not significantly change after three months of storage at 4°C in all liposomes with extract. Namely, the size and zeta potential of MLVs with extract were 3085 ± 60 nm and –25.8 ± 1.5 mV, respectively, while the diameter and zeta potential of both types of SUVs with extract were 154.1-261.0 nm and ~ –13 mV. The antioxidant activity of pure extract (diluted to achieve the same concentration as in liposomes) was also determined. The data of the measurements are presented in Table 1.

As can be seen from Table 1, MLVs showed significantly higher antioxidant capacity in both assays (12.02 ± 0.54 μmol TE/mL and 81.9 ± 0.4%, respectively) in comparison to sonicated vesicles (10.75-11.00 μmol TE/mL and 79.3-80.9%, respectively) and pure extract (11.37 ± 0.52 μmol TE/mL and 79.7 ± 0.5%, respectively). Namely, the higher antioxidant potential of the extract encapsulated in liposomes was expected due to the presence of antioxidants (added to the phospholipid mixture used by the producer) and phosphatidylcholine. The obtained results are in agreement with the literature data where was shown a slight antioxidant effect of plain liposomal particles that originated from synthetic antioxidant compounds already presented in phospholipids, as well as phosphatidylcholine (De Luca et al., 2022). At the same time, sonication has caused a decrease in the antioxidant potential of the liposomal samples (Table 1). The ultrasound probe can cause changes in the antioxidant capacity of the sample because of its potential to generate free radicals. Hence, sonication can damage natural antioxidants, particularly during extended exposure (Horžić et al., 2012). However, there was no statistically significant difference between the anti-ABTS effect of the samples treated by different amplitudes of ultrasound waves (45 and 75%) and times (15 and 30 min).

**Table 1.** Antioxidant potential of multilamellar and small unilamellar (sonicated) smoke tree extract-loaded liposomes (MLVs and SUVs, respectively) and pure extract.

| Sample | ABTS (μmol TE*/mL) | | neutralization of DPPH radicals (%) | |
|---|---|---|---|---|
| | 1st day | After 3 months | 1st day | After 3 months |
| MLVs | 12.02 ± 0.54$^{a}$ | 13.01 ± 0.18$^{a}$ | 81.9 ± 0.4$^{a}$ | 82.9 ± 1.0$^{a}$ |
| SUVs (15 min, 45% amplitude) | 10.75 ± 0.53$^{b}$ | 10.01 ± 0.29$^{b}$ | 80.9 ± 0.4$^{b}$ | 79.8 ± 0.9$^{b}$ |
| SUVs (30 min, 70% amplitude) | 11.00 ± 0.24$^{b}$ | 10.52 ± 0.48$^{b}$ | 79.3 ± 0.6$^{c}$ | 78.7 ± 1.1$^{b}$ |
| Extract | 11.37 ± 0.52$^{ab}$ | 12.03 ± 0.32$^{ab}$ | 79.7 ± 0.5$^{c}$ | 79.0 ± 0.7$^{b}$ |

*TE, Trolox equivalent; different letters in each column showed statistically significant difference ($p<0.05$, n=3, one-way ANOVA followed by Duncan's post hoc test)

Nevertheless, in the DPPH assay, a prolonged sonication period and a higher value of amplitude caused a significant drop in the antioxidant potential of smoke tree extract-loaded liposomes (Table 1). In addition, sonication of the liposomes with encapsulated compounds can result in a leakage of the entrapped components causing the reduction of liposome antioxidant activity. Considering that the two used antioxidant assays are based on various principles, reactions, and probes, and the measurements were performed at different pH values and wavelengths, the obtained data, as well as differences among them provide a good insight into the overall antioxidant potential of smoke tree extract-loaded multilamellar and small unilamellar liposomal particles. As can be seen from Table 1, the storage at 4 °C for three months did not cause significant changes in the antioxidant potential of the obtained liposomes confirming the protective role of liposomal particles on bioactive principles from smoke tree extract.

## 4. CONCLUSION

Smoke tree extract-loaded multilamellar and small (sonicated) unilamellar liposomal particles were prepared with the aim of providing better stability and bioavailability of the extract's bioactives, as well as their longer recovery. The multilamellar liposomal system with extract showed significantly higher antioxidant activity in both antioxidant tests compared to sonicated liposomes and pure extract. The application of prolonged sonication time and a higher amplitude resulted in significantly lower anti-DPPH activity of the liposomes, while the mentioned parameters did not significantly influence the ABTS radical scavenging potential of the samples. Future experiments should be focused on other biological properties of the obtained liposomes, including antimicrobial, anti-biofilm, anti-inflammatory, skin regeneration, and enzyme-inhibitory effects, as well as on monitoring the release of bioactive compounds in simulated skin conditions.

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## CONFLICT OF INTEREST

The authors declare that they have no financial or commercial conflict of interest.

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