UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE VICTOR BABEȘ | TIMIȘOARA

EUROTOX 2026 VIENNA | AUSTRIA 13–16 SEPTEMBER 2026

Cytotoxic and anti-clonogenic effects of a Taxus baccata aril–seed ethanolic extract in A549 lung cancer cells: an in vitro/in ovo assessment

Alex-Robert Jîjie [1,2], Elena-Alina Moacă [1,2], Diana Haj Ali [1,2], Alina Anton [1,2], Andreea Cristea [1,2], Ionela-Daliana Minda [3,4], Cristina Adriana Dehelean [1,2]

[1] University Clinic of Toxicology, Drug Industry, Management and Legislation, Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2nd Eftimie Murgu Square, 300041 Timisoara, Romania
[2] Research Centre for Pharmaco-Toxicological Evaluation, Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy, 2nd Eftimie Murgu Square, 300041 Timisoara, Romania
[3] University Department of Pharmacognosy, Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2nd Eftimie Murgu Square, 300041 Timisoara, Romania
[4] Research and Processing Center for Medicinal and Aromatic Plants, Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy Timisoara, 2nd Eftimie Murgu Square, 300041 Timisoara, Romania

Corresponding author: alex-robert.jijie@umft.ro

BACKGROUND

Taxus baccata L. is a pharmaceutically relevant species known for its taxane-related bioactivity. However, mixed extracts obtained from the aril–seed matrix remain poorly characterized, particularly regarding their cytotoxic potential and preclinical safety. Recent evidence indicating antiproliferative and pro-apoptotic activity of aril-derived constituents supports further investigation of this underexplored matrix [1–4].

MATERIALS & METHODS

- A549 cells: TBAS-EE 10–150 µg/mL, 24 h.
- Assays: MTT; bright-field microscopy; JC-1; Hoechst 33342/MitoTracker Red CMXRos; AO/PI; colony formation.
- In ovo: HET-CAM, TBAS-EE 150 µg/mL.

AIM & OBJECTIVES

✓ To investigate the cytotoxic and anti-clonogenic activity of TBAS-EE in A549 lung adenocarcinoma cells and its preliminary in ovo vascular tolerability.

RESULTS & DISCUSSIONS

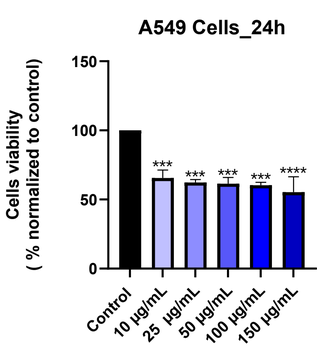


Figure 1. Viability of A549 cells after 24 h exposure to TBAS-EE (10–150 µg/mL), assessed by MTT assay and expressed relative to untreated control.

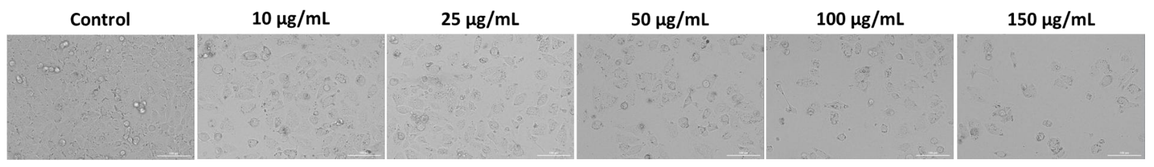


Figure 2. Bright-field microscopy of A549 cells after 24 h exposure to TBAS-EE (10–150 µg/mL), compared with untreated control.

Fig. 1: TBAS-EE significantly reduced A549 cell viability at all tested concentrations. Viability decreased to 65.53%, 62.33%, 61.45%, 61.31%, and 55.36% following treatment with 10, 25, 50, 100, and 150 µg/mL, respectively. Although relatively similar viability levels were observed across part of the concentration range, an overall reduction was evident, with the strongest cytotoxic effect occurring at 150 µg/mL. These findings demonstrate a pronounced acute cytotoxic effect of TBAS-EE on A549 cells.

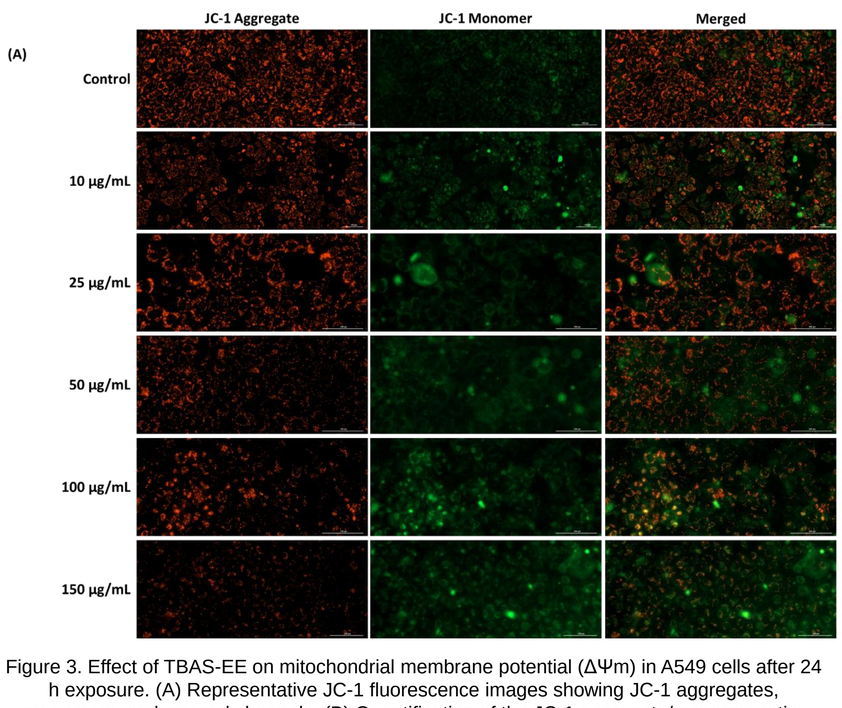


Figure 3. Effect of TBAS-EE on mitochondrial membrane potential (ΔΨm) in A549 cells after 24 h exposure. (A) Representative JC-1 fluorescence images showing JC-1 aggregates, monomers, and merged channels. (B) Quantification of the JC-1 aggregate/monomer ratio, expressed relative to untreated control.

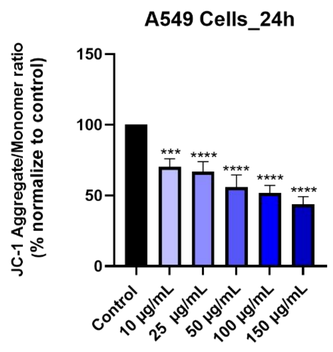


Fig. 2: Bright-field microscopy revealed progressive alterations in A549 cell morphology following TBAS-EE treatment. A reduction in cell confluence and loss of cell-to-cell contact were already observed at 10 µg/mL. Increasing concentrations resulted in more pronounced morphological changes, including cell shrinkage, membrane blebbing, and acquisition of a rounded morphology, consistent with cellular injury and cytotoxicity.

Fig. 3: JC-1 staining demonstrated a progressive loss of mitochondrial membrane potential following TBAS-EE exposure. Increasing extract concentrations produced a decrease in red fluorescence associated with polarized mitochondria and a concomitant increase in green fluorescence, indicative of mitochondrial depolarization. Quantitative analysis confirmed this effect, with the JC-1 aggregate/monomer ratio decreasing to 70.25%, 66.89%, 55.88%, 51.57%, and 43.79% at 10, 25, 50, 100, and 150 µg/mL, respectively. These findings indicate a concentration-dependent impairment of mitochondrial function and suggest that mitochondrial dysfunction is involved in TBAS-EE-induced cytotoxicity.

Fig. 4: Fluorescence microscopy revealed progressive alterations in both nuclear and mitochondrial morphology. Untreated cells exhibited predominantly regular nuclei and a well-organized mitochondrial network, whereas TBAS-EE-treated cells showed increasingly irregular nuclei with chromatin condensation, lobulation, and partial fragmentation. In parallel, the elongated and interconnected mitochondrial network progressively changed toward shortened, fragmented, rounded, and punctate structures, particularly at the highest concentrations. These findings further support mitochondrial injury and nuclear alterations associated with TBAS-EE-induced cell death.

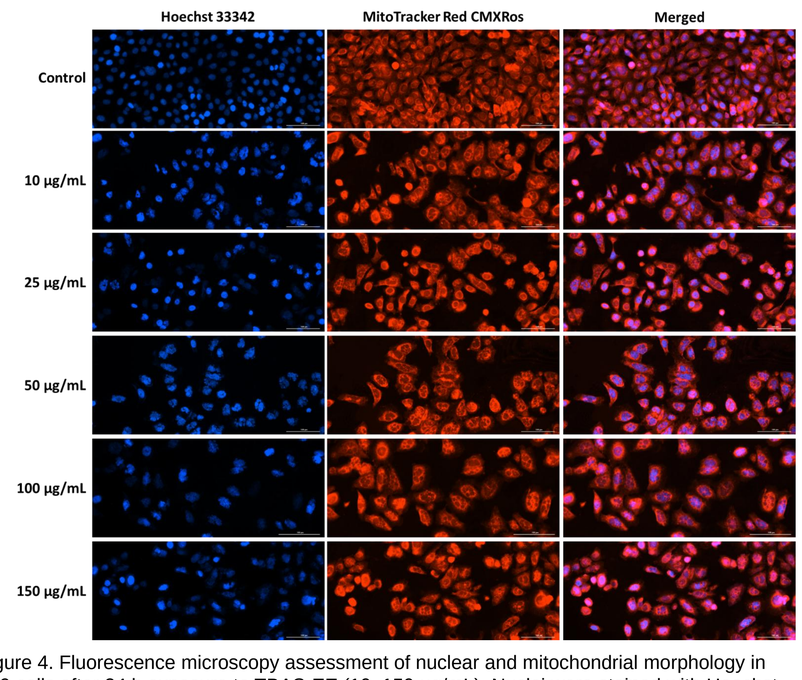


Figure 4. Fluorescence microscopy assessment of nuclear and mitochondrial morphology in A549 cells after 24 h exposure to TBAS-EE (10–150 µg/mL). Nuclei were stained with Hoechst 33342 and mitochondria with MitoTracker Red CMXRos; merged images illustrate their combined cellular distribution.

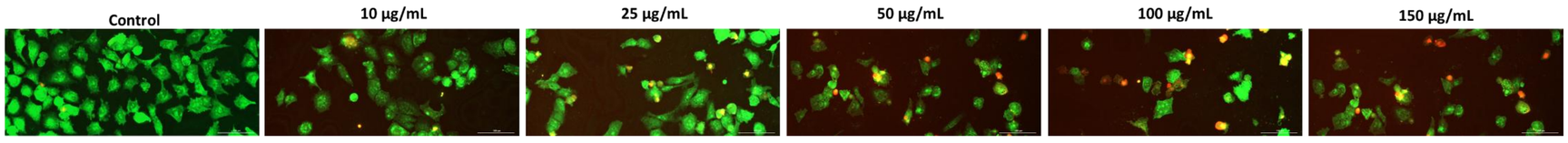
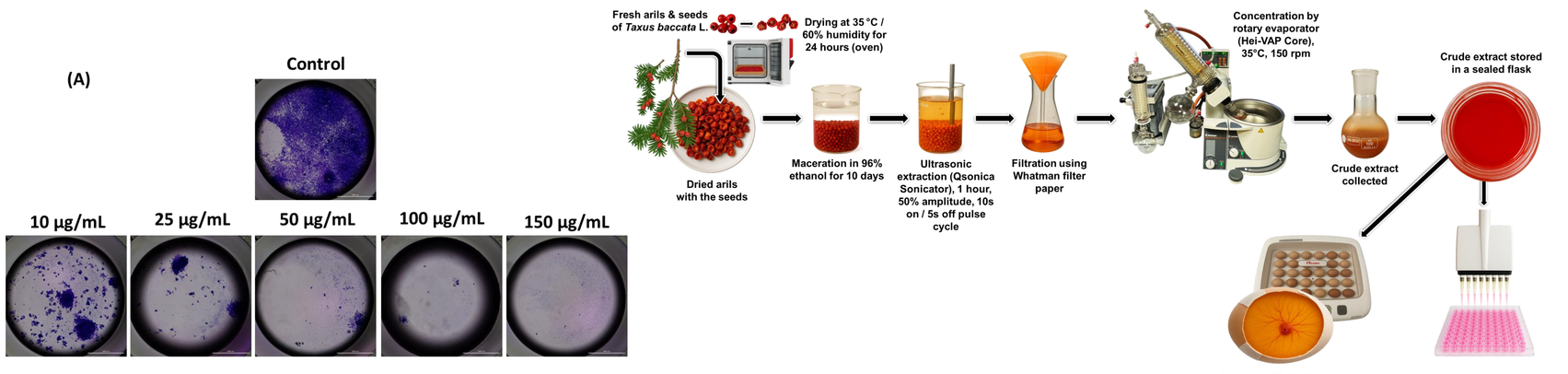


Figure 5. Acridine orange/propidium iodide (AO/PI) double staining of A549 cells following 24 h exposure to TBAS-EE (10–150 µg/mL), showing concentration-dependent changes associated with apoptotic and necrotic cell death.

Fig. 5: AO/PI staining revealed progressive changes in cell viability and morphology following TBAS-EE treatment. Control cells were predominantly viable and displayed green fluorescence, whereas treated cells increasingly exhibited features compatible with early apoptosis, including chromatin condensation and membrane blebbing. At higher concentrations, an increased number of orange/red PI-positive cells was observed, reflecting loss of plasma membrane integrity and suggesting progression toward late apoptosis and/or necrosis. Overall, the findings indicate a mixed apoptotic/necrotic pattern of TBAS-EE-induced cell death.



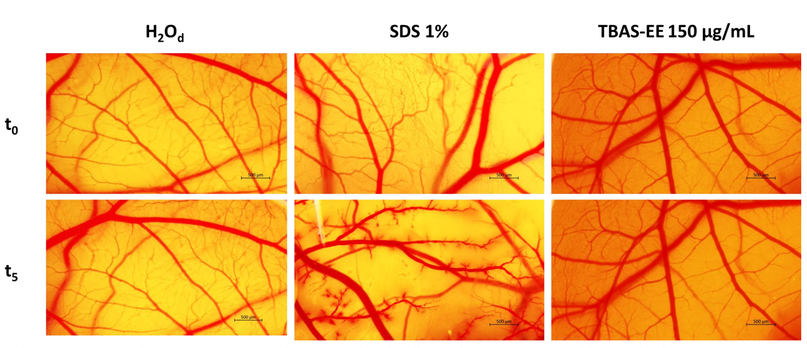


Figure 7. HET-CAM assay showing the chorioallantoic membrane (CAM) before treatment (t_0) and after 5 min (t_5) following application of TBAS-EE (150 µg/mL), compared with negative (H_2Od) and positive (SDS 1%) controls.

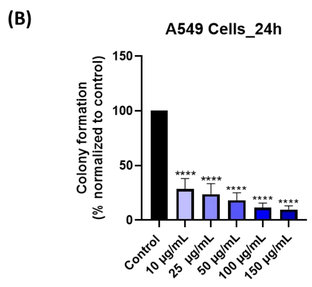


Fig. 6: TBAS-EE produced a pronounced concentration-dependent inhibition of A549 colony formation. A substantial reduction in clonogenic capacity was already evident at 10 µg/mL, while cells exposed to the highest concentration almost completely lost their ability to form colonies. Colony formation decreased to 28.39%, 23.79%, 18.64%, 11.45%, and 9.63% following treatment with 10, 25, 50, 100, and 150 µg/mL, respectively. These results demonstrate that the effect of TBAS-EE extends beyond acute cytotoxicity, producing a marked and persistent impairment of long-term proliferative capacity.

Figure 6. Effect of TBAS-EE on the clonogenic capacity of A549 cells. (A) Representative images of crystal violet-stained colonies following exposure to increasing TBAS-EE concentrations. (B) Quantitative analysis of colony formation expressed relative to untreated control.

Fig. 7: Application of TBAS-EE at 150 µg/mL produced no visible signs of hemorrhage, vascular lysis, or coagulation during the observation period. The CAM response remained comparable to the negative control (H_2Od), whereas the positive control (SDS 1%) produced evident vascular irritation. These findings indicate that TBAS-EE was non-irritant and well tolerated at the vascular level in ovo under the tested experimental conditions, despite its pronounced cytotoxic activity against A549 tumor cells.

CONCLUSIONS

✓ TBAS-EE exerted a pronounced cytotoxic effect against A549 human lung adenocarcinoma cells, significantly reducing cell viability after 24 h exposure.
✓ Treatment induced marked changes in cellular morphology and a progressive loss of mitochondrial membrane potential, accompanied by disruption of the mitochondrial network.
✓ Nuclear alterations, including chromatin condensation and fragmentation, together with AO/PI staining, supported the occurrence of a mixed apoptotic/necrotic cell death pattern.
✓ TBAS-EE strongly impaired the long-term clonogenic capacity of A549 cells, with colony formation decreasing to below 10% at the highest tested concentration.
✓ Despite its cytotoxic activity in A549 cells, TBAS-EE at 150 µg/mL showed no vascular irritant effects in the HET-CAM assay, indicating good preliminary in ovo tolerability.
✓ Collectively, these findings support further mechanistic investigation and broader safety assessment of TBAS-EE as a potential source of bioactive compounds with anticancer relevance.

REFERENCES

- Jîjie AR, Iliescu D, Sbârcea L, Boru C, Pătrașcu D, Iftode OA, et al. A Deep Dive into the Botanical and Medicinal Heritage of Taxus. Plants. 2025;14:1439.
- Tabaszewska M, Antoniewska A, Rutkowska J, Skoczylas Ł, Słupski J, Skoczeń-Słupska R. Bioactive Components, Volatile Profile and In Vitro Antioxidative Properties of Taxus baccata L. Red Arils. Molecules. 2021;26:4474.
- Dumitraș DA, Bunea A, Vodnar DC, Hanganu D, Pall E, Cenariu M, et al. Phytochemical Characterization of Taxus baccata L. Aril with Emphasis on Evaluation of the Antiproliferative and Pro-Apoptotic Activity of Rhodoxanthin. Antioxidants. 2022;11:1039.
- Dumitraș D, Dreanca AI, Pall E, Gal AF, Rus V, Morohoschi AG, et al. Inhibition of Tumor Growth and Modulation of Antioxidant Activity of Rhodoxanthin Isolated from Taxus baccata Aril against B16F10 Murine Malignant Melanoma. Antioxidants. 2022;11:2264.

ACKNOWLEDGEMENT

- This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-IV-P2-2.1-TE-2023-1941, within PNCDI IV; by the Doctoral School of the "Victor Babeș" University of Medicine and Pharmacy of Timișoara; and by the DOCTOMED project, Cod SMIS 350965.