Toxicological profiling and biosafety evaluation of a Taxus baccata aril ethanolic extract

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BACKGROUND

Taxus baccata L. (European yew) is a well-known gymnosperm whose bark and needles contain diterpenoid alkaloids used in oncology. However, the aril, the fleshy red appendage surrounding the seed, is the only non-toxic part of the plant and has been traditionally considered safe. Recent research has revealed that the aril is rich in bioactive compounds, including carotenoids (e.g., rhodoxanthin), flavonoids, polyphenols, and mucilages, which confer antioxidant, anti-inflammatory, and anticancer properties [1,2]. These characteristics highlight the aril's underexplored value as a safe and bioactive matrix suitable for topical formulations.

AIM & OBJECTIVES

The present study aimed to evaluate the biosafety of an ethanolic extract obtained from *Taxus baccata* arils (TX_A) as a potential candidate for topical formulations.
Specific objectives were:

- ✓ To assess the *in vitro* cytotoxicity, morphology, and nuclear integrity of epidermal cells exposed to TX_A.
- ✓ To determine the irritant potential of TX_A using a 3D reconstructed human epidermis model (EpiDerm™).
- ✓ To evaluate the *in vivo* dermal tolerance of TX_A in a murine model by monitoring skin barrier parameters (TEWL, erythema, hydration).
- ✓ To investigate the vascular irritancy of TX_A through the *in ovo* HET-CAM assay.

RESULTS & DISCUSSIONS

$$IS = 5 \times \left[\frac{301 - Sec\ H}{300}\right] + 7 \times \left[\frac{301 - Sec\ L}{300}\right] + 9 \times \left[\frac{301 - Sec\ C}{300}\right]$$

non-irritant (**0-0.9**), weak irritant (**1-4.9**), moderately irritant (**5-8.9**), strongly irritant (**9-21**)

Sample / Concentration	Time (seconds)			Irritation score (IS)
	hemorrhage (t_H)	lysis (t_L)	coagulation (t_C)	
H_2O_d	300	300	300	0.070
SLS 1%	14	50	20	18.070
TX_A 50 µg/mL	300	300	300	0.070
TX_A 100 µg/mL	300	300	300	0.070
TX_A 200 µg/mL	300	300	300	0.070

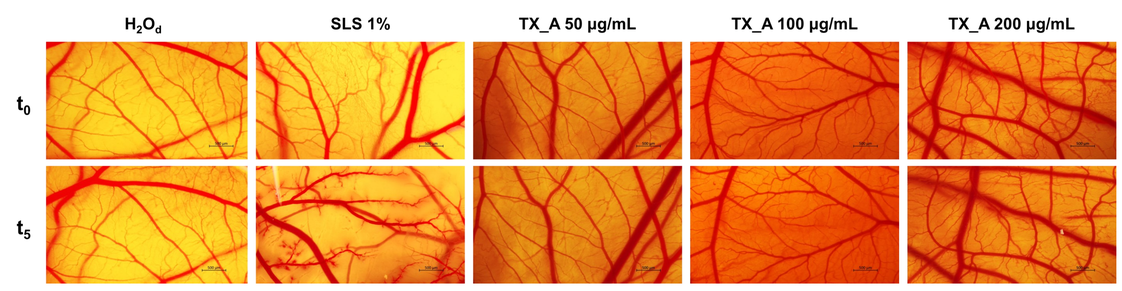


Figure 1. HET-CAM assay showing CAM responses before and after treatment with TX_A (50–200 µg/mL) compared to negative (H_2O) and positive (SLS 1%) controls. Scale bars represent 200 µm.

Fig. 1: Application of TX_A (50–200 µg/mL) on the chorioallantoic membrane (CAM) produced no signs of vascular damage, such as hemorrhage, lysis, or coagulation, within the observation period. Irritation scores were comparable to the negative control (H_2O), whereas the positive control (SLS 1%) induced strong vascular responses. These findings confirm the extract's excellent biocompatibility and absence of irritant potential *in ovo*.

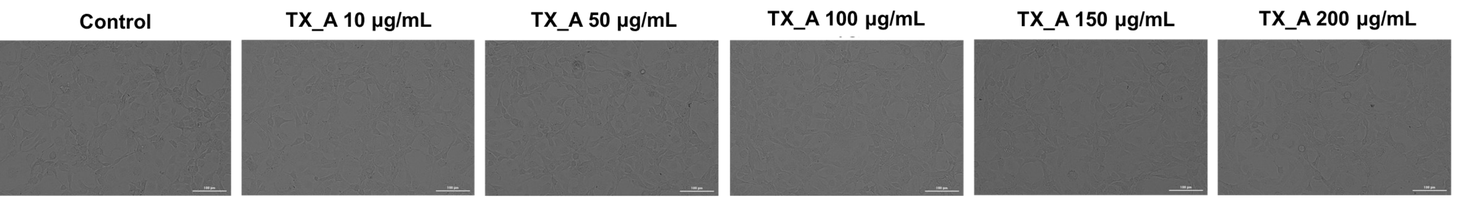


Figure 2. Morphology of JB6 Cl 41-5a cells 24 h after TX_A treatment (10–200 µg/mL), showing preserved normal cell shape.

Fig. 2: Bright-field microscopy revealed that TX_A treatment (10–200 µg/mL) did not induce visible alterations in cell morphology. Cells retained their typical elongated, adherent shape, with no evidence of rounding, detachment, or dysmorphology. These results reinforce the viability data and indicate that the extract does not impair cell structure.

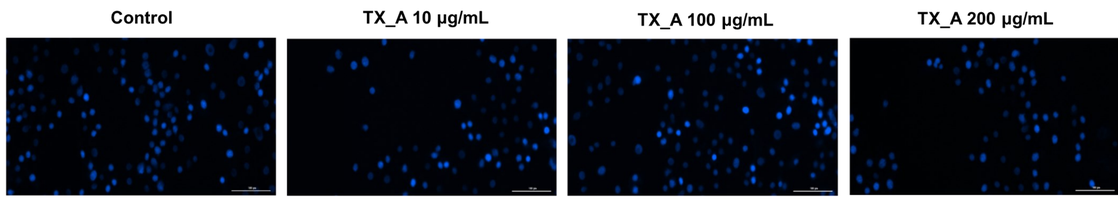


Figure 3. Nuclear morphology of JB6 Cl 41-5a cells stained with Hoechst 33342 after 24 h TX_A exposure (10–200 µg/mL).

Fig. 3: Fluorescence analysis with Hoechst 33342 showed that nuclei of TX_A-treated cells preserved their normal morphology across all tested doses. No chromatin condensation, nuclear fragmentation, or apoptotic bodies were detected compared to the control. This supports the conclusion that the extract does not trigger genotoxic or apoptotic responses under the tested conditions.

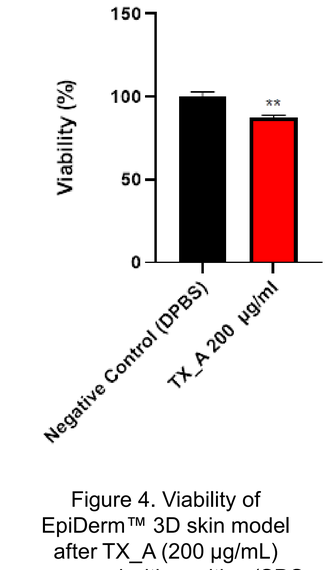


Figure 4. Viability of EpiDerm™ 3D skin model after TX_A (200 µg/mL) compared with positive (SDS 5%) and negative (DPBS) controls.

Fig. 4: Treatment of reconstructed human epidermis (EpiDerm™) with TX_A (200 µg/mL) maintained tissue viability at 87.55%, well above the OECD cut-off for classification as irritant. The negative control (DPBS) confirmed normal viability, while the positive control (SDS 5%) reduced viability to ~5%, validating assay sensitivity. These data demonstrate that the extract is non-irritant in a human-relevant in vitro model.

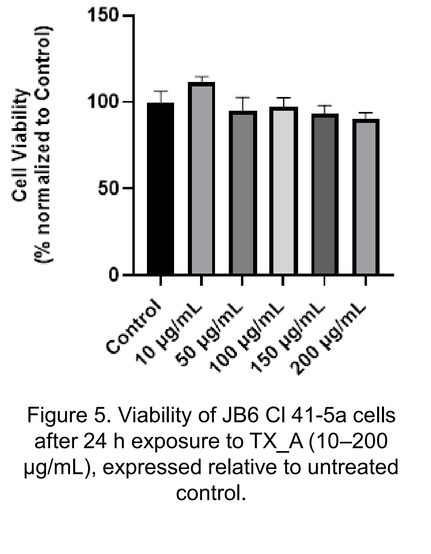
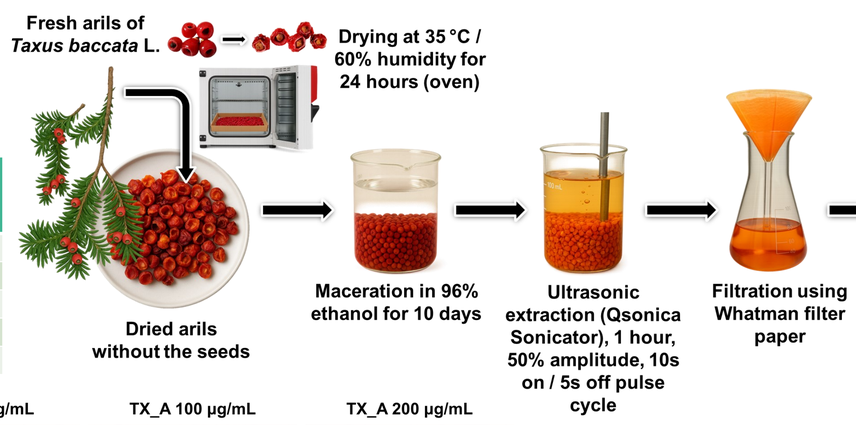


Figure 5. Viability of JB6 Cl 41-5a cells after 24 h exposure to TX_A (10–200 µg/mL), expressed relative to untreated control.

Fig. 5: Exposure of JB6 Cl 41-5a cells to TX_A for 24 h maintained viability above 90% even at the highest concentration tested (200 µg/mL). Interestingly, a mild stimulatory effect was observed at the lowest dose (10 µg/mL), suggesting potential proliferative or protective cellular responses. The absence of cytotoxicity indicates good in vitro biosafety of the extract.

Topical application of TX_A (1 mg/mL, every 3 days for 15 days) on nude mice did not cause significant changes in transepidermal water loss (TEWL), erythema, or hydration parameters. All values remained within physiological ranges, and animals exhibited no adverse behavior or weight loss. The results confirm the dermal safety and tolerance of the extract under repeated application conditions.

MATERIALS & METHODS



***In vivo*, BALB/c nude mice: 50 µL extract (1 mg/mL) applied topically every 3 days for 15 days; TEWL, erythema, hydration monitored**

***In ovo* – HET-CAM assay: 600 µL extract applied on CAM, observed for 5 min for irritation (hemorrhage, lysis, coagulation)**

***In vitro* screening: JB6 Cl 41-5a murine epidermal cells exposed to 10–200 µg/mL extract**
- ✓ **Viability via MTT assay**
- ✓ **Morphology via bright-field microscopy**
- ✓ **Nuclear integrity via Hoechst 33342**

EpiDerm – 3D reconstructed epidermis: extract applied on tissue surface; viability measured to evaluate skin irritation potential

CONCLUSIONS

- ✓ The ethanolic extract from *Taxus baccata* arils (TX_A) was shown to be ***non-toxic***, ***non-irritant***, and ***well-tolerated*** across complementary *in vitro*, *in vivo*, and *in ovo* models.
- ✓ JB6 Cl 41-5a cells preserved viability, morphology, and nuclear integrity after exposure to TX_A.
- ✓ EpiDerm™ 3D skin model confirmed the absence of irritant effects, as tissue viability remained ~87.5%, well above the internationally accepted cut-off of 50% for skin irritation.
- ✓ HET-CAM assay and murine dermal application further demonstrated the lack of vascular irritation and excellent skin tolerance.
- ✓ Collectively, these findings support the biosafety of *T. baccata* aril extract and encourage its further exploration as a safe, antioxidant-rich matrix for pharmaceutical and dermatological applications.

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