Biosynthesis efficiency comparison of *in vitro* culture systems for producing botanicals from plant stem cells of *Aralia racemosa* L. for cosmetic applications

Kiełkiewicz R.[1], Obrębski M.[2], Śliwińska A.[1], Skowrońska W.[1], Sykłowska-Baranek K.[1]

[1] Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
[2] MicrobiotaLab, Department of Pharmaceutical Microbiology and Bioanalysis, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
E-mail address: rafal.kielkiewicz@wum.edu.pl (Kiełkiewicz R.)

1 INTRODUCTION

Aralia racemosa L. (American spikenard), known in traditional North American medicine for its anti-inflammatory and skin-soothing properties [1], has been used for centuries to treat various skin conditions such as eczema, inflammation, burns, itching, and wounds [2]. Despite its therapeutic relevance, this species remains phytochemically poorly characterized, and its field cultivation is constrained by slow growth and low germination rates. The genus *Aralia* is particularly notable for its pharmacologically relevant saponins and phenolic derivatives, which contribute to diverse biological activities (Fig. 1) [3]. Plant biotechnology offers a sustainable alternative by establishing stem cell-derived systems – callus (CC) and cell suspension (SC) cultures for the controlled production of bioactive metabolites, independent of environmental limitations [4]. Such biotechnological platforms are increasingly recognized as promising sources of secondary metabolites for cosmetic applications, where the demand for antioxidant and skin-protective compounds is rapidly growing.

To our knowledge, this is the first study to establish and characterize *in vitro* CC and SC of *A. racemosa*, comparing their growth dynamics, metabolite production, and antioxidant activity. A novel productivity parameter, biosynthetic efficiency per inoculum (BEI), was applied to standardize the assessment across different culture systems.

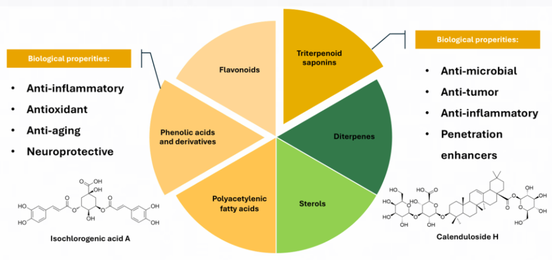


Fig. 1. Main groups of pharmacologically relevant secondary metabolites reported in the genus *Aralia* [3].

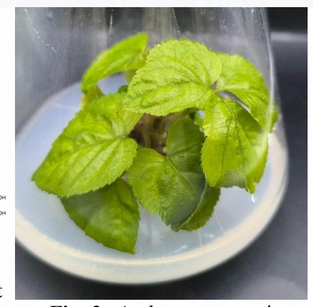


Fig. 2. *Aralia racemosa* in *in vitro* culture.

2 MATERIALS AND METHODS

Leaf explants from *Aralia racemosa* plantlets (Fig. 2) were cultured on Murashige and Skoog (MS) medium [5] supplemented with 1.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) to establish CC and SC (Fig. 3). Biomass growth was monitored over a 50-day period under controlled conditions, including measurements of fresh and dry weight (FW, DW), medium pH, conductivity, and sucrose concentration.

Methanolic extracts from plantlet: shoots (AR-Sh), rhizomes (AR-Rh), roots (AR-Ro), CC (AR-C_{35}), and SC (AR-S_{40}) in stationary phase, were analyzed for total phenolic (TPC), flavonoid (TFC), and saponin (TSC) contents, and for antioxidant capacity using FRAP and DPPH assays, according to Kiełkiewicz et al. [6]. Specialized metabolites were profiled and quantified by UHPLC-DAD-ESI-MS^3, with emphasis on triterpenoid saponins and phenolic acids (chlorogenic acid, isochlorogenic acid A, calenduloside H, calendulaglycoside A).

Cytotoxicity of extracts was assessed using the MTT assay on normal human keratinocytes (HaCaT) and malignant melanoma cells (HTB-140), with camptothecin applied as a positive control.

To compare culture productivity across systems and timepoints, a new parameter – Biosynthetic Efficiency per Inoculum (BEI) – was introduced, defined as compound yield per gram of fresh inoculum:

$$\textbf{Biosynthetic efficiency per inoculum}\left(\frac{mg}{g}\,\mathbf{FW_I}\right) = \frac{\textbf{Metabolite content}\left(\frac{\mathbf{mg}}{\mathbf{g}}\,\mathbf{DW}\right)\ \textbf{x Dry biomass yield (g DW)}}{\textbf{Fresh inoculum mass (g FW}_I\textbf{)}}$$

Integrates growth and metabolite yield – combines biomass accumulation with metabolite concentration, providing a realistic measure of biosynthetic performance.

Standardizes comparisons across systems – allows direct evaluation of callus and suspension cultures, which differ in growth kinetics and cannot be reliably compared using only mg/L or mg/g DW values.

Identifies optimal harvest phases – highlights the timepoints when metabolite production per inoculum reaches its maximum, guiding efficient culture maintenance and collection.

Improves assessment of productivity – moves beyond simple metabolite content, capturing the true biosynthetic efficiency of a culture relative to its starting material.

BIOSYNTHETIC EFFICIENCY PER INOCULUM INDEX

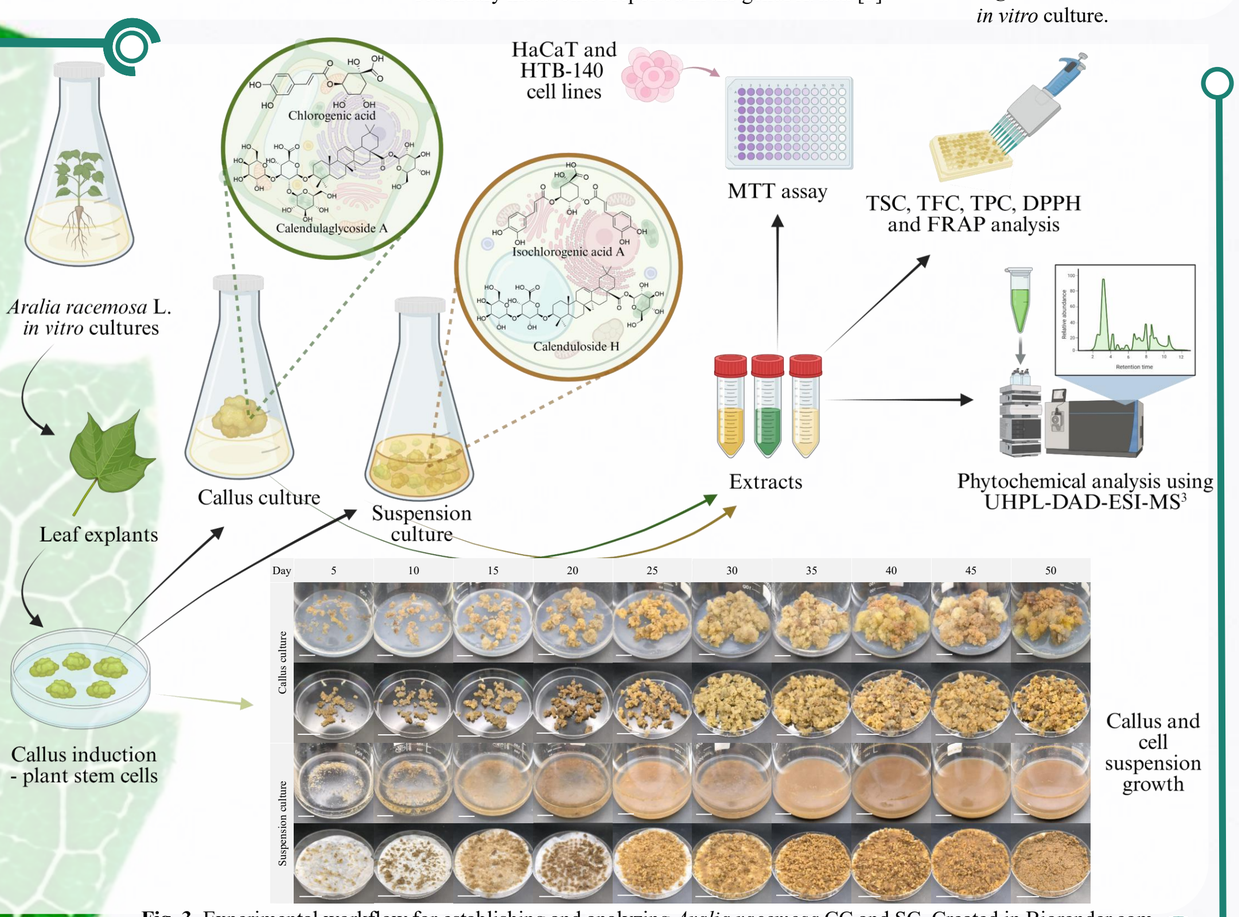


Fig. 3. Experimental workflow for establishing and analyzing *Aralia racemosa* CC and SC. Created in Biorender.com

3 RESULTS

I. Both CC and SC of *A. racemosa* exhibited succesive growth phases (lag, exponential, linear, deceleration, stationary and decline) over 50 days, accompanied by dynamic changes in pH, conductivity, and sucrose consumption (Fig. 4). Metabolite profiling revealed the presence of triterpenoid saponins and phenolic acids, with chlorogenic acid, isochlorogenic acid A, calenduloside H, and calendulaglycoside A as key compounds.

II. Total phenolic, flavonoid, and saponin contents, as well as antioxidant activity (FRAP, DPPH), were substantial in both systems (Fig. 5 A – F). The application of BEI highlighted differences in biosynthetic efficiency between CC and SC and identified optimal harvest phases not evident from conventional units (Fig. 5 G – J).

III. UHPLC-MS^3 chromatograms revealed that the majority of specialized metabolites remained accumulated within biomass of SC, while only minor amounts were released into the culture medium, indicating that SC primarily act as intracellular metabolite reservoirs (Fig. 6).

IV. Extracts from both culture types showed low cytotoxicity toward HaCaT keratinocytes, while demonstrating a more selective inhibitory effect against HTB-140 melanoma cells (IC_{50} values summarized in Table 1).

Table 1. IC_{50} values for *A. racemosa* extracts in human skin keratinocytes (HaCaT) and melanoma cells (HTB-140) after 24 h exposure, determined by MTT assay. IC_{50} is defined as the concentration of extract required to reduce cell viability by 50 %. Extracts were derived from callus (AR-C_{35}), and suspension cultures (AR-S_{40}). Camptothecin was used as a positive control.

	HaCaT	HTB-140
AR-C_{35}	1320.92 ± 529.69 µg/mL	795.88 ± 284.72 µg/mL
AR-S_{40}	712.73 ± 98.74 µg/mL	711.42 ± 177.19 µg/mL
Camptothecin	42.22 ± 2.16 µg/mL	76.06 ± 19.99 µg/mL

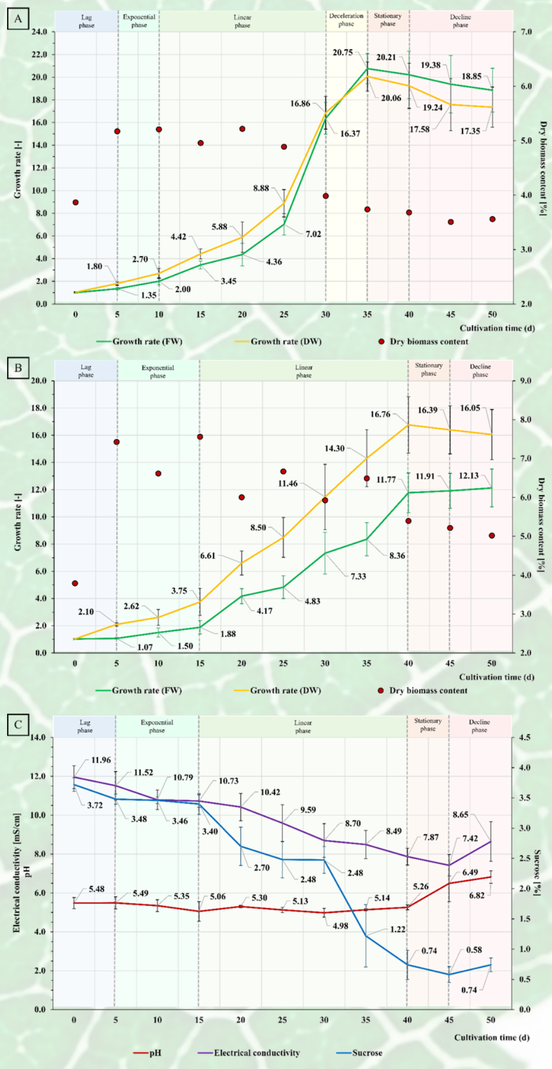


Fig. 4. (A) Callus growth kinetics of *A. racemosa* cultured on solid MS medium supplemented with 1.0 mg L^{-1} 2,4-D; (B) Cell suspension growth kinetics of *A. racemosa* cultured in liquid MS medium supplemented with 1.0 mg L^{-1} 2,4-D; changes in growth rate based on fresh (FW) and dry biomass (DW), and dry biomass content (%) during 50 d of cultivation. (C) Changes in pH, electrical conductivity [mS cm^{-1}], and sucrose concentration [%] liquid medium. Distinct growth phases are indicated.

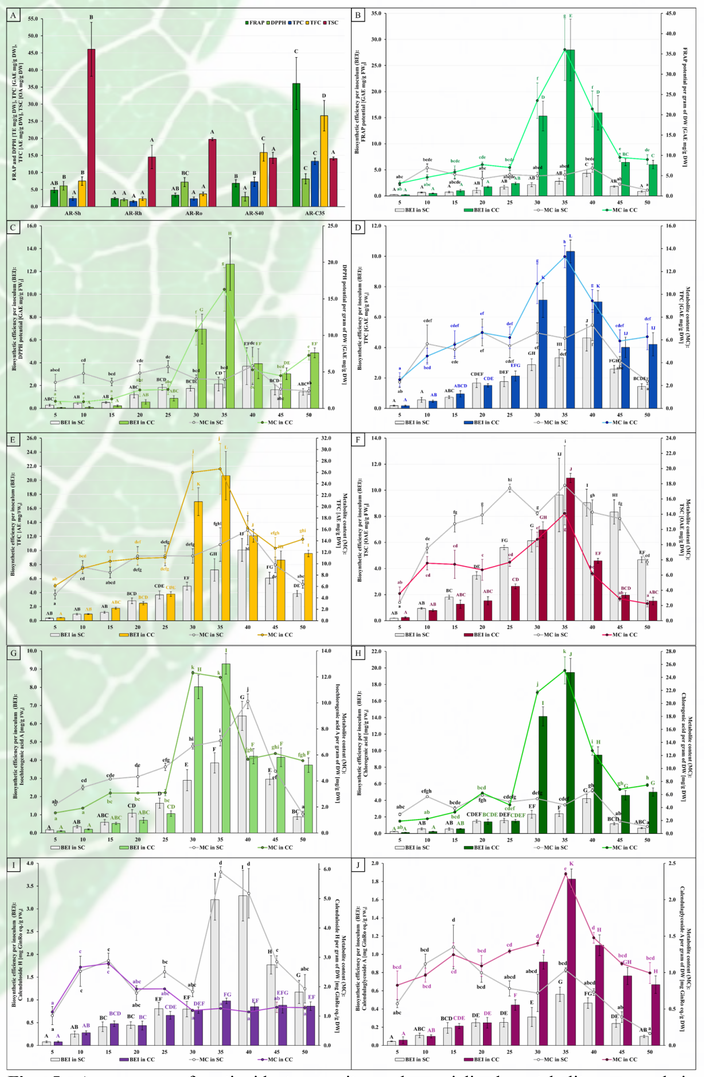


Fig. 5. Assessment of antioxidant capacity and specialized metabolite accumulation in *A. racemosa in vitro* cultures. (A) TPC, TFC, and TSC and antioxidant activity (FRAP, DPPH) in extracts from shoots, rhizomes, roots, SC, and CC. (B–F) Time-course dynamics of metabolite content (MC, lines) and biosynthetic efficiency per inoculum (BEI, bars) in CC and SC for FRAP, DPPH, TPC, TFC, and TSC. (G–J) Time-course dynamics of selected metabolites (isochlorogenic acid A, chlorogenic acid, calenduloside H, calendulaglycoside A) expressed as MC (lines) and BEI (bars). Different letters indicate statistically significant differences (Tukey's HSD, $p < 0.05$). (GAE – gallic acid equivalent; AE – apigenin equivalent; OAE – oleanolic acid equivalent; TE – Trolox equivalent; DW – dry weight; FWI – fresh weight of inoculum).

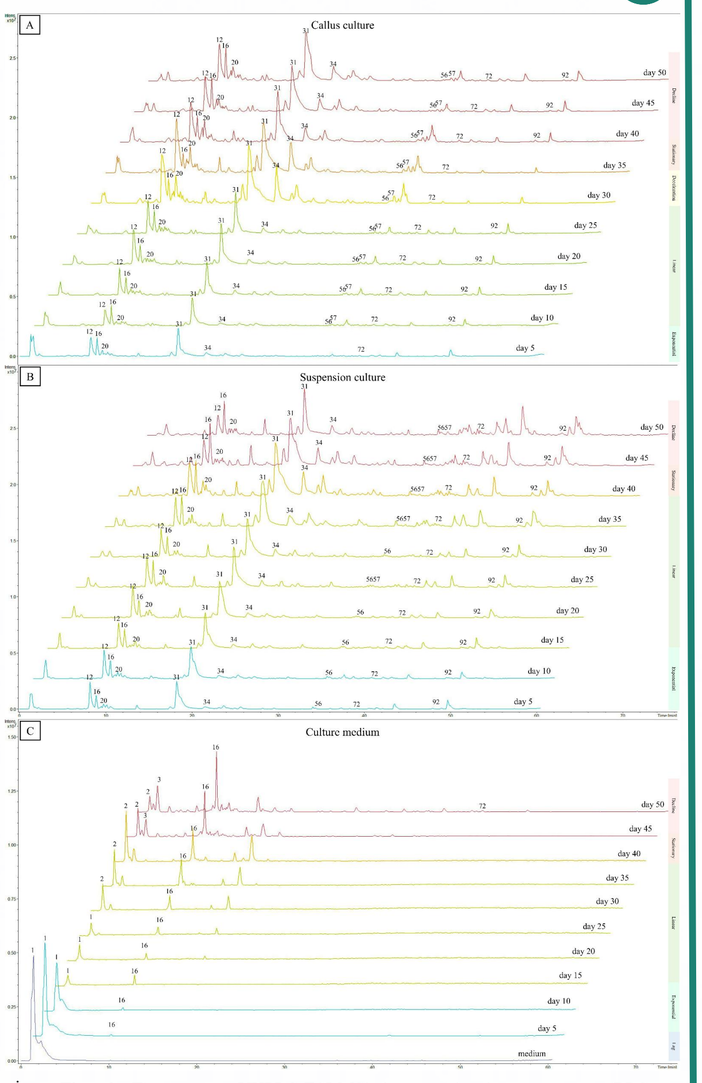
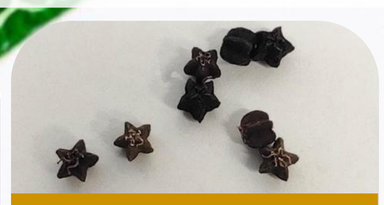


Fig. 6. Time-course UHPLC-ESI-MS^3 base peak chromatograms (BPCs, negative ion mode) of methanolic extracts from *A. racemosa* callus (A), cell suspension (B), and post-culture medium (C), recorded over 50 d of cultivation. Sampling was performed every 5 d. Distinct growth phases are indicated. 1 – sucrose, 2 – gluconic acid, 3 – quinic acid, 12 – chlorogenic acid, 16 – apigenin arabinoside, 20 – 4-*O*-*p*-coumaroylquinic acid, 31 – isochlorogenic acid A, 34 – isochlorogenic acid C, 56 – (5-5')/(8'-O-4")-dehydrotriferulic acid, 57 – calendulaglycoside A, 72 – calenduloside H, 92 – calenduloside E.

4 CONCLUSIONS

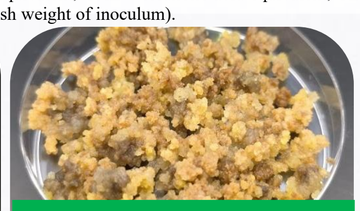
Plant stem cell systems – callus and suspension cultures of *A. racemosa* were successfully established for the first time as novel plant stem cell platforms.

Controlled metabolite production – these cultures provide a sustainable and standardized source of bioactive compounds, independent of environmental limitations.



Skin-beneficial phytochemicals – cultures accumulated triterpenoid saponins and phenolic acids with recognized antioxidant and protective properties for skin care.

High antioxidant activity – extracts showed strong radical-scavenging capacity, supporting their potential in anti-aging and protective formulations.



Intracellular metabolite retention – most metabolites were kept in biomass, simplifying downstream processing and supporting industrial use in cosmetics.

Selective safety – extracts were well tolerated by skin keratinocytes but more toxic to melanoma cells, indicating safety for skin and potential anti-cancer relevance.

Industrial perspective – BEI optimizes culture efficiency, enabling large-scale production of plant stem cell extracts for the cosmetic industry.

5 REFERENCES

1) Prasanth, D.; Rao, A.; Yejella, R. Exploration of Anti-nociceptive and Anti-inflammatory Activities of Metha-nolic extract of *Aralia racemosa* L. Root. Curr Res Complement Altern Med: CRCAM-104 2017; **2)** Quattrocchi, U., CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology. London CRC Press Inc.: 2012; p 3960; **3)** Clement, J.A., Clement, E.S., 2015. The medicinal chemistry of genus *Aralia*. Curr Top Med Chem 14, 2783-2801; **4)** Wawrosch, C.; Zotchev, S. B., Production of bioactive plant secondary metabolites through *in vitro* technologies-status and outlook. Appl Microbiol Biotechnol 2021, 105, (18), 6649-6668; **5)** Murashige, T.; Skoog, F., A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum 1962, 15, (3), 473-497; **6)** Kiełkiewicz, R.M., Obrębski, M., Śliwińska, A.A., Równicki, M., Kawka, M., Sykłowska-Baranek, K., Granica, S., 2024. Detailed qualitative and quantitative UHPLC-DAD-ESI-MS3 analysis of *Aralia spinosa* L. (Araliaceae) phytochemical profile to evaluate its potential as novel plant material for bioactive compounds acquisition using *in vitro* culture. Industrial Crops and Products 2024 219; 119123.

Natural Cosmetics International Meeting