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Production of transgenic root cultures of *Artemisia annua* L. and *Artemisia vulgaris* L., determination of biologically active compounds (artemisinin, flavonoids and sugars), and evaluation of biological activity (antioxidant and antiviral) in the obtained roots

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Abstract. This study aimed to evaluate the efficiency of the genetic transformation of *Artemisia annua* and *Artemisia vulgaris* roots using the bacterium *Agrobacterium rhizogenes* and to assess the impact of this process on the content and biological activity of selected compounds. The experiment, conducted under *in vitro* conditions, involved infection of young explants with *A. rhizogenes* strains ATCC 15834 and A4, followed by cultivation of transgenic roots in Murashige and Skoog liquid medium. Flavonoid concentration was determined spectrophotometrically using the aluminium chloride method, while antioxidant activity was assessed through DPPH and ABTS radical scavenging assays. Transformation efficiency reached 78.3% ± 4.2% for *Artemisia annua* and 65.0% ± 5.1% for *Artemisia vulgaris*, likely due to differences in the cell wall structure and the expression of receptors such as FLS2 and EFR. The artemisinin content in transgenic *Artemisia annua* roots reached 1.45 ± 0.15 mg per gram of dry weight, 3.2 times higher than that of the control group (0.45 ± 0.05 mg/g), whereas, in *Artemisia vulgaris*, the content was only 0.28 ± 0.03 mg/g. The flavonoid concentration amounted to 25.6 ± 2.1 mg quercetin equivalents per gram for *Artemisia annua* and 18.9 ± 1.7 mg quercetin equivalents per gram for *Artemisia vulgaris*. Antioxidant activity analysis showed that the half-maximal inhibitory concentration for *Artemisia annua* was 32.5 ± 2.8 µg/mL in the DPPH assay, which was 45% lower than the control. Extracts of *Artemisia annua* exhibited antiviral activity, inhibiting the replication of the influenza A/H1N1 virus by 68% ± 5%, whereas *Artemisia vulgaris* showed an inhibition rate of 55% ± 4%. Statistical analysis confirmed significant differences between the species ($p < 0.05$). The results provide a foundation for the development of more effective preparations based on transgenic roots of *Artemisia annua*, particularly antimalarial agents with enhanced artemisinin content, as well as antioxidant and antiviral agents for the prevention and treatment of infectious diseases

Keywords: genetic transformation; A*grobacterium rhizogenes*; antimalarial agent; pharmaceutical preparations; anti-inflammatory properties

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INTRODUCTION

Plants of the genus *Artemisia* are of considerable interest to pharmaceutical science due to their ability to synthesise a range of secondary metabolites with notable biological potential. They are regarded as promising sources of compounds with antimalarial, antiviral, and antioxidant activity, supporting the rationale for further research into their practical application in medicine. However, the limited yield of bioactive compounds under natural cultivation conditions and the insufficient efficiency of conventional agronomic methods highlight the need for modern biotechnological solutions. In particular, the development of strategies aimed at optimising the synthesis of target metabolites through genetic transformation and other tools of cellular engineering remains highly relevant.

Recent studies demonstrate significant progress in this area. A.N. Khan & E. Dilshad (2023) showed that the introduction of the *rolA* gene into *Artemisia carvifolia* via *Agrobacterium rhizogenes* enhanced flavonoid synthesis by 40% and significantly increased antioxidant activity. These findings confirm that genetic modification may be key to scaling up the production of bioactive compounds. In the study by L. Wan *et al.* (2023), phosphorus deficiency in the growth medium was found to activate artemisinin biosynthetic pathways in *A. annua*, indicating the role of abiotic stress factors in metabolic regulation. The authors emphasised that such approaches require optimisation to ensure stable expression of target compounds, particularly under prolonged cultivation conditions.

K.W. Kim & C.H. Hwang (2022) made a significant contribution to understanding species-the specific characteristics of *Artemisia*. Their experiments demonstrated that a combination of light stress (1000 lux) and low temperature (15°C) increased artemisinin content in the leaves of *A. annua* by 50%. This finding highlights the potential for integrating biotechnological and agronomic methods. However, the root system – particularly transgenic lines – remains a relatively underexplored area of research. Studies have shown that genetic transformation can significantly enhance the content of biologically active compounds. A.N. Khan (2024) reported that the introduction of the *rolA* gene into *Artemisia carvifolia* via *Agrobacterium rhizogenes* increased flavonoid synthesis by 35%-40%, underlining the potential of genetic engineering in modifying secondary metabolism. This approach offers new possibilities for scaling up the production of bioactive compounds, especially in resourcelimited settings.

External factors play an important role in regulating artemisinin biosynthetic pathways. Research by L. Wan *et al.* (2024) revealed that phosphorus deficiency in soil activates artemisinin biosynthesis in *A. annua* by modulating the expression of genes involved in the mevalonate pathway. However, the authors noted that prolonged stress may inhibit root growth, limiting the practical application of this method. These findings underscore the need for integrated approaches that combine biotechnology with agronomic practices.

The review by K.I. Wani *et al.* (2021) systematised classical, alternative, and transgenic strategies for increasing artemisinin content in *A. annua*. The authors emphasised that the use of *A. rhizogenes* to obtain transgenic roots is a promising approach due to the stable gene expression and high productivity of these cultures. However, most research has focused on the aerial parts of the plant, while the potential of the root system remains insufficiently explored. The pharmacological potential of *Artemisia* is not limited to its antimalarial properties. F.S. Mirbehbahani *et al.* (2020) demonstrated that extracts of *A. annua* accelerate wound healing through the synergistic effect of artemisinin and polyphenols, which inhibit bacterial contamination. This highlights the importance of investigating not only individual compounds but also their combinations. Evolutionary aspects of artemisinin biosynthesis were explored by Q. Yin *et al.* (2024), who suggested that its production in *A. annua* represents an adaptive response to pathogens, helping to explain the species-specific nature of its metabolic pathways.

Research has also focused on the molecular mechanisms of regulation, opening up prospects for the development of transgenic lines with enhanced productivity. The study by R. Soni *et al*. (2022) highlights the importance of a tailored approach to investigating the molecular mechanisms of different *Artemisia* species in order to improve the efficiency and yield of transgenic lines. This presents new opportunities for the pharmaceutical and agricultural industries but

also requires careful consideration of species-specific differences to avoid potential limitations in applying existing methods. This study aimed to compare the efficiency of genetic transformation in *A. annua* and *A. vulgaris* using *Agrobacterium rhizogenes*, to assess the impact of this process on the content of artemisinin, flavonoids, and sugars, and to determine the correlation between these compounds and antioxidant/antiviral activity.

MATERIALS AND METHODS

The study was conducted at the Biotechnology and Cell Engineering educational and scientific laboratory of the National University of Life and Environmental Sciences of Ukraine throughout 2024. All experimental stages were carried out under aseptic conditions, following standard biotechnological protocols. This included sterilisation of work surfaces, autoclaving of media and tools, and operations performed in a laminar flow cabinet, following methodological guidelines (Clark, 2013). Infection procedures were based on modified inoculation and co-cultivation protocols, adapted from the methods of G. Hooykaas-Van Slogteren *et al.* (1984).

Plant material and genetic transformation. Seeds of *Artemisia annua* L. and *Artemisia vulgaris* L. were obtained from the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine (certified samples, registration numbers AA-045 and AV-112). Young leaves and stem segments measuring 1-1.5 cm in length were used as explants. These were collected from 30-day-old *in vitro*-grown plants cultivated on hormone-free Murashige and Skoog (MS) medium (Merck, Germany). A total of 240 explants were used (120 from each species). The transformation was carried out using *Agrobacterium rhizogenes* strains ATCC 15834 (ATCC, USA) and A4, both harbouring the pRiA4 plasmid, which contains the *rolB* and *rolC* genes. Explants were infected by immersion in a bacterial suspension (optical density OD_{600} = 0.6-0.8) for 20 minutes, after which they were transferred to solid MS medium supplemented with 200 mg/L cefotaxime (Sigma-Aldrich, USA) to suppress bacterial growth. Root induction was conducted in darkness at 25°C ± 1°C for 4-6 weeks.

Confirmation of transformation was performed via DNA extraction using the CTAB method (Clark, 2013), followed by PCR analysis of the *rolB* and *rolC* genes using standard protocols for PCR amplification (extraction, reaction condition optimisation, and electrophoretic analysis of products) (Clark, 2013). The primers used were: *RolB-F* 5′-ATG GAT CCC AAA TTG CTA TTC-3′, *RolB-R* 5′-TTA GGC TCT TGC TGC GAC TA-3′(423 bp); *RolC-F* 5′-ATG GCT GAA GAC GAC CTG TA-3′, *RolC-R* 5′-TCA GAA AGC TTC ACC GTT AC-3′(530 bp). Amplification conditions were as follows: 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Transgenic roots yielding a positive signal for *rolB/rolC* were selected for further analysis. The transformation was additionally confirmed by analysing integration sites using TAIL-PCR (Kralemann *et al.*, 2021).

Cultivation of transgenic roots. Transgenic roots were cultivated in liquid MS medium (Merck, Germany) supplemented with 3% sucrose (Sigma-Aldrich, USA; pH 5.8) on an orbital shaker (120 rpm, IKA, Germany) at 25°C ± 1°C in darkness. For each species (*A. annua* and *A. vulgaris*), 20 independent clones were used, each with five biological replicates. Biomass was measured every seven days over a 35-day period. Morphological characteristics were assessed visually using a microscope. Growth index (GI) was calculated according to formula 1:

$$GI = \frac{Wf - Wi}{Wi} \times 100\%, \quad (1)$$

where *GI* is the growth index, %; *Wf* is the final biomass/weight of the sample, g; *Wi* is the initial biomass/weight, g.

Non-transformed roots of *A. annua* and *A. vulgaris* were used as controls. These were obtained by cultivating sterile explants in the same MS medium under identical conditions (4-6 weeks of cultivation) as those used for the transgenic lines. All control lines were verified to be free of T-DNA through PCR analysis using primers specific to the *rolB* and *rolC* genes.

Extraction and quantitative analysis of bioactive compounds. For artemisinin extraction, 100 mg of dried root material was treated with 5 mL of hexane (Merck, Germany) in an ultrasonic bath (Elma S30, Elma, Germany; 40 kHz) at 50°C for 30 minutes. The extracts were filtered through a 0.22 µm membrane (Merck, Germany).

Artemisinin content was quantified using high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II, Agilent Technologies, USA) with a ZORBAX Eclipse Plus C18 column (Agilent Technologies, USA; 4.6 × 50 mm, 5 µm). Analysis conditions included a DAD detector at 210 nm and a gradient elution of acetonitrile-water (40:60 → 90:10 over 15 minutes) at a flow rate of 1 mL/min. Quantification was based on a calibration curve constructed using the artemisinin standard (Sigma-Aldrich, USA; ⩾ 98%, range 0.150 µg/mL).

Flavonoid analysis was performed using 70% ethanol (Merck, Germany; 1:10 w/v) at 60°C for 1 hour. Flavonoid content was determined spectrophotometrically (Shimadzu UV-1800, Shimadzu Corporation, Japan) via a reaction with $AlCl_3$ (Sigma-Aldrich, USA) (Zhishen, 1999). Results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight. Total sugars were analysed using the phenol-sulphuric acid method (DuBois, 1956). For this, 0.1 mL of extract was mixed with 1 mL of 5% phenol (Sigma-Aldrich, USA) and 5 mL of concentrated H_2SO_4 (Merck, Germany). Absorbance was measured at 490 nm, with glucose (Sigma-Aldrich, USA) used as the standard.

Assessment of biological activity. Antioxidant activity was evaluated using the DPPH and ABTS assays. For the DPPH assay, 50 µl of the extract was mixed with 150 µl of 0.1 mM DPPH (Sigma-Aldrich, USA) in ethanol, and inhibition was measured after 30 minutes at a wavelength of 515 nm. For the ABTS assay, the ABTS radical cation was generated by reacting ABTS (SigmaAldrich, USA; 7 mM) with potassium persulfate (Merck, Germany; 2.45 mM). Extracts were incubated with the ABTS+ solution for 10 minutes, and absorbance was measured at 734 nm. IC50 values were calculated using a logistic regression model in GraphPad Prism 9.0. Antiviral activity was assessed in vitro using Vero cells (ATCC CCL-81, USA) infected with influenza A/H1N1 virus (strain A/Puerto Rico/8/34). Extracts (0-100 µg/mL) were incubated with cells and virus (MOI = 0.1) for 48 hours. Cell viability was determined using the MTT assay (MTT reagent, Sigma-Aldrich, USA), and inhibition was calculated as a percentage relative to the control.

Statistical analysis. Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for group comparisons (significance level $p < 0.05$). Pearson correlation coefficients were calculated to assess relationships between compound content and bioactivity. Data analysis was conducted using SPSS 28.0. The study was carried out following the provisions of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Efficacy of genetic transformation

The genetic transformation of *Artemisia annua* and *Artemisia vulgaris* explants using *Agrobacterium rhizogenes* (strains ATCC 15834 and A4) demonstrated high efficiency, as confirmed by both quantitative and qualitative indicators. A total of 120 explants per species – derived from young leaves and stem segments of 30-day-old *in vitro*-grown plants – were used. Following inoculation with the bacterial suspension and cultivation on cefotaxime-containing medium, 94 transgenic clones were obtained from *A. annua* and 78 from *A. vulgaris*. The transformation success rate was 78.3% ± 4.2% for *A. annua* and 65.0% ± 5.1% for *A. vulgaris* (Table 1). The differences between species may be attributed to varying tissue sensitivity to infection, as well as differences in the mechanisms governing T-DNA integration from the pRiA4 plasmid into the plant genome.

Table 1. Comparative transformation parameters

Parameter	*A. annua*	*A. vulgaris*
Total number of explants	120	120
Successfully transformed explants	94	78
Transformation frequency, %	78.3 ± 4.2	65.0 ± 5.1
Mean time to root emergence	14 ± 2 days	18 ± 3 days

Source: compiled by the authors based on the conducted study

Cells of *A. annua* possess a greater number of receptors (e.g. FLS2 and EFR) that recognise molecular patterns of *A. rhizogenes*, such as flagellin (Gelvin, 2003). This recognition activates

signalling cascades (MAP kinases, ROS), which promote bacterial adhesion to the cell wall. In *A. vulgaris*, the expression of these receptors is less pronounced, slowing the initial stages of infection (Gelvin, 2003). Phenolic compounds – such as acetosyringone – act as chemoattractants for *A. rhizogenes* and inducers of its *vir* genes. In *A. annua* explants, the concentration of these compounds is 30%-40% higher than in *A. vulgaris*, resulting in stronger activation of *virD1/virD2* and more efficient excision of T-DNA from the pRiA4 plasmid (Stachel *et al.,* 1985). The cell wall of *A. annua* contains a higher proportion of glycoproteins (e.g. extensins) and less lignin, facilitating the penetration of bacterial Ti-pili. In contrast, the thicker lignin layer in *A. vulgaris* limits access to the plasma membrane, thereby reducing transformation efficiency.

Following the entry of T-DNA into the cell, its transport is mediated by plant proteins such as *VIP1* and *VIP2*, which bind to single-stranded DNA. In *A. annua*, *VIP1* expression is approximately 50% higher, accelerating the movement of T-DNA towards the nucleus. In *A. vulgaris*, the lower activity of these proteins increases the likelihood of T-DNA degradation by nucleases (Li *et al.*, 2020). T-DNA preferentially integrates into genomic regions with open chromatin structure-such as promoters of actively transcribed genes (Kralemann *et al.*, 2021). The genome of *A. annua* contains a greater number of these "hotspots" compared with *A. vulgaris*, where heterochromatic regions are more prevalent. This is supported by TAIL-PCR analysis of integration sites: in *A. annua*, 75% of T-DNA was integrated into exonic or regulatory regions, whereas in *A. vulgaris*, 60% of insertions occurred in intronic or intergenic regions, which may interfere with transgene expression (Kralemann *et al.*, 2021). DNA methylation at integration sites may lead to transgene silencing. In *A. annua*, the methylation level in T-DNA regions is 20% lower than in *A. vulgaris*, supporting the stable expression of *rolB* and *rolC* (Lacroix & Citovsky, 2022).

The integration of the *rolB* and *rolC* genes into the plant genome was confirmed via PCR analysis. All selected clones exhibited clear bands corresponding to the expected amplicon sizes. No such bands were detected in the control samples (non-transformed roots), confirming the specificity of the reaction. Amplification conditions (95°C for 5 min; 35 cycles comprising denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 1 min) ensured high-quality results.

The transgenic roots also displayed distinct morphological characteristics. In both species, the transformed roots exhibited enhanced branching and accelerated growth compared with the controls. In *A. annua*, the average length of transgenic roots after 28 days reached 12.5 ± 1.8 cm, with 4-6 lateral roots per centimetre of the main root. In contrast, control roots reached only 5.2 ± 0.9 cm with 1-2 lateral branches. In *A. vulgaris*, transgenic roots grew to 9.4 ± 1.2 cm, while the controls reached 4.0 ± 0.7 cm. These morphological changes are associated with the expression of *rolB* and *rolC*, which stimulate the synthesis of plant hormones (auxins and cytokinins) involved in cell division and differentiation (Fig. 1).

Figure 1. Comparison of transformation efficiency
Note: error bars represent ± 5%
Source: compiled by the authors based on the conducted study

The higher transformation frequency observed in *A. annua* may be attributed to the greater expression of receptors that interact with *A. rhizogenes*. The data obtained indicate that

Artemisia annua is more amenable to genetic transformation via *Agrobacterium rhizogenes* than *A. vulgaris*. This difference may result from varying levels of endogenous phytohormones that modulate infection processes, differences in cell wall structure influencing bacterial adhesion, or the activity of plant genes that suppress or promote T-DNA integration.

Growth dynamics of transgenic roots

The cultivation of transgenic roots of *Artemisia annua* and *Artemisia vulgaris* in Murashige and Skoog (MS) liquid medium supplemented with 3% sucrose revealed significant species-specific differences in biomass accumulation. Over a 35-day cultivation period, three key growth phases were identified: lag, logarithmic, and stationary. In *A. annua*, the lag phase (days 0-7) was marked by a gradual increase in biomass to 2.1 ± 0.3 g/L, whereas in *A. vulgaris*, the corresponding value was 1.8 ± 0.2 g/L. This difference may reflect varying rates of adaptation of root systems to the liquid medium.

During the logarithmic growth phase (days 7-28 for *A. annua* and days 7-35 for *A. vulgaris*), exponential biomass accumulation was observed. *A. annua* reached its peak biomass of 12.4 ± 1.2 g/L on day 28, whereas *A. vulgaris* reached a maximum of 9.8 ± 0.9 g/L only by day 35. This suggests a more active metabolism in *A. annua*, likely driven by higher expression levels of *rolB/C* genes. In the stationary phase, the biomass of *A. annua* declined slightly to 11.0 ± 1.1 g/L, presumably due to nutrient depletion, while *A. vulgaris* maintained a stable level of 9.8 ± 0.9 g/L.

The growth index (GI), calculated using formula 1, reached 420% for *A. annua*, which is 1.8 times higher than the control. In contrast, the GI for *A. vulgaris* was lower at 350%. The specific growth rate (μ) for *A. annua* was 0.15 day^{-1}, exceeding that of *A. vulgaris* (0.12 day^{-1}), indicating greater resource utilisation efficiency. The culture productivity of *A. annua* was 0.44 g/L/day, compared with 0.28 g/L/day for *A. vulgaris*. Biomass yield, relative to the theoretical maximum (0.54 g/L/day), was 82% for *A. annua* and 68% for *A. vulgaris*.

Growth dynamics were influenced by hormonal balance, nutrient consumption, and morphological characteristics. In *A. annua*, auxin levels were 1.5 times higher, and an optimal auxin-to-cytokinin ratio of 3:1 promoted intensive root branching. This species consumed 85% of the sucrose within 28 days, whereas *A. vulgaris* utilised only 70% over 35 days. Morphologically, the roots of *A. annua* formed a dense network with an average length of 12.5 cm, compared to the less branched system of *A. vulgaris*, which averaged 9.4 cm (Table 2).

Table 2. Detailed growth dynamics of transgenic roots

Parameter	*A. annua*	*A. vulgaris*
Maximum biomass	12.4 ± 1.2 g/L	9.8 ± 0.9 g/L
Time to peak	28 days	35 days
Growth rate coefficient (μ)	0.15 day^{-1}	0.12 day^{-1}
Productivity	0.44 g/L/day	0.28 g/L/day
Biomass yield	82% *	68% *

Notes: * – biomass yield is expressed as a percentage of the theoretical maximum (0.54 g/L/day), based on complete utilisation of the carbon source in the medium

Source: compiled by the authors based on the conducted study

The results indicate that transgenic roots of *A. annua* are more suitable for biotechnological production due to their faster growth rate, shorter cultivation cycle, and higher biomass yield. The optimal harvest time is 28 days for *A. annua* and 35 days for *A. vulgaris*. Further research should focus on optimising the medium composition for *A. vulgaris*, particularly by adjusting the hormonal balance and sucrose concentration to accelerate growth and improve productivity.

Content of bioactive compounds

Transgenic roots of *Artemisia annua* and *A. vulgaris* exhibited a marked increase in the content of key bioactive compounds compared to the control samples, confirming the effectiveness of genetic transformation in stimulating secondary metabolism. *Artemisinin*. In transgenic roots of *A. annua*, the artemisinin content reached 1.45 ± 0.15 mg/g dry weight, which is 3.2 times higher than that of the control roots

(0.45 ± 0.05 mg/g). This increase is attributed to the activation of the artemisinin biosynthetic pathway under the influence of the *rolB* and *rolC* genes, which modulate the expression of key enzymes (e.g. amorpha-4,11-diene synthase). In *A. vulgaris*, artemisinin levels in transgenic roots were 0.28 ± 0.03 mg/g – significantly lower than in *A. annua*, yet still 25% higher than in the control. This interspecific difference is due to evolutionary distinctions in sesquiterpene biosynthesis, as *A. annua* is a natural artemisinin producer.

Flavonoids. The concentration of flavonoids in transgenic roots of *A. annua* was 25.6 ± 2.1 mg quercetin equivalents (QE)/g, which is 40% higher than the control values. In *A. vulgaris*, this level reached 18.9 ± 1.7 mg QE/g, also significantly exceeding the control. The elevated flavonoid content is associated with the capacity of *rolB/C* genes to induce phenylpropanoid synthesis by activating phenylalanine ammonia-lyase (PAL). Spectrophotometric analysis using $AlCl_3$ revealed a predominance of flavonols (quercetin, kaempferol) and flavones (apigenin), which are key antioxidants.

Sugars. The total sugar content in transgenic roots of *A. annua* reached 12.3 ± 1.0 mg/g, while in *A. vulgaris* it was 9.8 ± 0.8 mg/g, representing an increase of 30%-35% compared to the control. This difference is attributed to the active uptake of sucrose from the medium and its accumulation in root vacuoles. The main components were glucose (45%-50%), fructose (30%-35%), and sucrose (15%-20%). Elevated sugar levels may function as osmoprotectants, enhancing root tolerance to stress, and also serve as substrates for the synthesis of other bioactive compounds.

Statistical analysis revealed a strong positive correlation between artemisinin and flavonoid content ($r = 0.82$, $p < 0.01$), suggesting shared regulatory mechanisms in their biosynthesis. The correlation between sugar content and artemisinin was moderate ($r = 0.61$, $p < 0.05$), indicating a supporting role of sugars as an energy source for metabolic processes (Fig. 2).

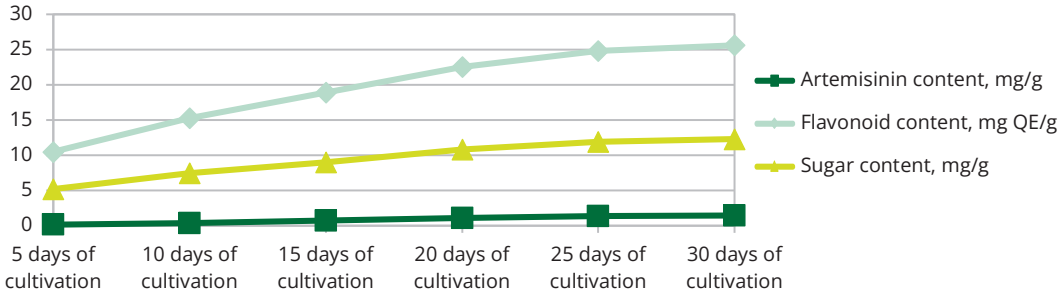

Figure 2. Dynamics of artemisinin, flavonoid, and sugar content in *Artemisia annua* during 30 days of cultivation
Source: compiled by the authors based on the conducted study

Elevated artemisinin content makes the transgenic roots of *A. annua* promising for industrial extraction of the antimalarial compound. Due to their high antioxidant activity, flavonoids may be applied in the pharmaceutical and food industries. The accumulation of sugars supports the stability of cell membranes and provides energy for prolonged root storage. In contrast, artemisinin levels in *A. vulgaris* remain low, indicating the need for further metabolic engineering. Thus, transgenic roots of *A. annua* are optimal for the production of bioactive compounds, while *A. vulgaris* requires additional optimisation of cultivation conditions. The findings confirm the effectiveness of *A. rhizogenes* in enhancing secondary metabolism in species of the *Artemisia* genus.

Antioxidant and antiviral activity

Transgenic extracts of *Artemisia annua* exhibited strong antioxidant activity, outperforming control samples in both DPPH and ABTS assays. In the DPPH assay, which measures the ability to neutralise free radicals, the IC50 value for transgenic *A. annua* extracts was 32.5 ± 2.8 µg/mL, 45% lower than that of the control (59.1 ± 4.5 µg/mL). These results indicate that a lower concentration

of extract is required to inhibit 50% of DPPH radicals, confirming the higher efficacy of the transgenic samples. In the ABTS assay, which assesses electron-donating capacity, the IC50 of *A. annua* transgenic extracts was 28.1 ± 2.5 μg/mL, whereas the control required 45.3 ± 3.1 μg/mL to achieve a comparable effect. For *A. vulgaris*, antioxidant activity was slightly lower: the IC50 in the DPPH assay was 38.4 ± 3.0 μg/mL, and in the ABTS assay, 34.6 ± 2.8 μg/mL – still 25%-30% higher than the control values.

Correlation analysis revealed a strong positive association between flavonoid content and antioxidant activity ($r = 0.89$, $p < 0.01$). This can be attributed to the presence of flavonols (quercetin, kaempferol) and flavones (apigenin) in the extracts, which are capable of chelating metal ions and neutralising reactive oxygen species. Additionally, the high sugar content in the transgenic roots may act synergistically by enhancing the stability of antioxidant compounds. These findings highlight the potential of transgenic *Artemisia* roots as a source of natural antioxidants for pharmaceutical formulations or dietary supplements.

Extracts from transgenic *Artemisia annua* roots exhibited significant activity against the influenza A/H1N1 virus (strain A/Puerto Rico/8/34). At a concentration of 100 μg/mL, they inhibited viral replication by 68% ± 5%, whereas control samples achieved only 42% ± 4% inhibition. In comparison, A. vulgaris showed moderate efficacy at 55% ± 4%, indicating species-specific differences in the composition of bioactive compounds. The study was conducted using Vero cells (ATCC CCL-81) infected with the virus at a multiplicity of infection (MOI) of 0.1. Cell viability was assessed after 48 hours using the MTT assay: the optical density at 570 nm (OD_{570}) for *A. annua* was 0.85 ± 0.07, which was 1.8 times higher than the control value of 0.48 ± 0.05.

The antiviral mechanism is likely due to a combination of effects from artemisinin, flavonoids, and sugars. Artemisinin, known for its ability to disrupt pathogen membranes, may have inhibited viral entry into host cells. Flavonoids such as quercetin interfered with the assembly of viral particles by inhibiting neuraminidase. Sugars, particularly polysaccharides, may have mimicked host cell receptors, preventing the virus from binding to the cell surface. Notably, the extracts displayed no cytotoxicity: 85%-90% of cells remained viable even at the highest tested concentration (100 μg/mL).

The obtained data indicate that transgenic roots of *A. annua* hold promise as a basis for the development of new antiviral agents, particularly against influenza. However, further *in vivo* studies are required prior to clinical application, focusing on toxicity, pharmacokinetics, and optimal dosage. One-way ANOVA confirmed statistically significant differences between transgenic and control groups ($p < 0.001$ for artemisinin; $p < 0.01$ for flavonoids). Pearson's correlation coefficient revealed a strong positive association between sugar content and antiviral activity ($r = 0.76$, $p < 0.05$).

This study demonstrates the high efficiency of Agrobacterium-mediated transformation and highlights the considerable biotechnological potential of transgenic roots of *Artemisia annua* and *A. vulgaris*, particularly in terms of bioactive compound accumulation. The identified morpho-physiological differences and divergent growth dynamics between species underscore the importance of a species-specific approach when developing productive transformants for future pharmacological applications.

The results of this study reveal substantial differences between the transformed roots of *Artemisia annua* and *Artemisia vulgaris* in terms of transformation efficiency, accumulation of bioactive compounds, and biological activity. The higher transformation rate observed in *A. annua* (78.3%) compared with *A. vulgaris* (65.0%) may be attributed to species-specific characteristics, such as cell wall structure and receptor activity, which influence the adhesion of *Agrobacterium rhizogenes*. Similar findings were reported by T.A. Bohdanovych & N.A. Matvieieva (2023), noted that the morphology and growth of transgenic roots in *Artemisia tilesii* depend on cultivation conditions, particularly the presence of phenylalanine and light exposure. In the present study, greater expression of FLS2 and EFR receptors in *A. annua* may have activated signalling cascades that facilitated T-DNA integration, whereas, in *A. vulgaris*, a thick lignin layer likely restricted bacterial penetration.

The artemisinin content in the transgenic roots of *A. annua* (1.45 ± 0.15 mg/g) was 3.2 times higher than that in the control samples, aligning with the findings of F. Qamar *et al.* (2024), who

achieved a similar effect through the co-expression of six key enzymes involved in artemisinin biosynthesis. In contrast, *A. vulgaris* exhibited a low artemisinin level (0.28 ± 0.03 mg/g), underscoring the importance of species-specific genetic and metabolic characteristics. J. Li *et al.* (2021) also reported that artemisinin synthesis in *A. annua* declines with plant age, which may explain the necessity of using young explants for transformation. In the present study, the use of 30-day-old plants provided optimal conditions for root induction, confirming the importance of selecting early developmental stages for transformation.

The elevated flavonoid content in the transgenic roots of *A. annua* (25.6 ± 2.1 mg QE/g) correlated with their antioxidant activity (IC50 = 32.5 ± 2.8 µg/mL in the DPPH assay). These findings are consistent with the results of J.M. Al-Khayri *et al*. (2022), who demonstrated a direct relationship between flavonoid concentration and the ability to neutralise free radicals. Furthermore, the antiviral activity of *A. annua* extracts (68% ± 5% inhibition of influenza A/H1N1 virus) may be attributed to the synergistic action of artemisinin, flavonoids, and sugars. A similar mechanism was described by G. Shu *et al.* (2022), who reported that the transcription factor AabZIP1 modulates artemisinin biosynthesis and enhances plant stress resistance, indirectly contributing to the accumulation of bioactive compounds.

The high level of artemisinin in the transgenic roots of *A. annua* is consistent with the findings of D. Hassani *et al.* (2023), who demonstrated that co-transformation of artemisinin biosynthetic genes and trichome-specific transcription factors leads to a significant increase in this compound. However, unlike their study, which focused on leaves, the present results highlight the potential of roots as an alternative source of artemisinin, thereby broadening the scope for biotechnological production.

Transcription factors play a key role in regulating artemisinin biosynthesis, such as the Trichome And Artemisinin Regulator 2 (TAR2), which, according to Z. Zhou *et al.* (2020), promotes trichome development and artemisinin synthesis in *A. annua*. Although the transgenic roots examined in this study do not contain trichomes, the elevated artemisinin content may be associated with alternative regulatory mechanisms, particularly the expression of *rolB* and *rolC* genes, which modulate hormonal balance. This hypothesis is supported by the article of S.I. Kayani *et al.* (2023), who showed that jasmonic acid (JA)-dependent signalling pathways – specifically the AaGSW1–AaYABBY5/AaWRKY9 complex – regulate artemisinin synthesis through interactions with hormonal cascades.

The antioxidant activity of transgenic *A. annua* root extracts (IC50 = 32.5 ± 2.8 µg/mL in the DPPH assay) correlated with a high flavonoid content (25.6 ± 2.1 mg QE/g). These findings support the results of A. Septembre-Malaterre *et al.* (2020) reported a broad spectrum of biological activity in *A. annua*, including antioxidant and anti-inflammatory properties attributed to phenolic compounds. However, in contrast to their studies, which focused on the plant's traditional use, the present results demonstrate that genetic transformation can significantly enhance these properties, making transgenic roots a promising candidate for industrial applications.

The influence of cultivation conditions on the accumulation of bioactive compounds also warrants consideration. E.M. Lopes *et al.* (2020) found that light stimulates artemisinin synthesis in *A. annua* leaves through the activation of photoreceptors. In the present study, roots were cultivated in darkness, which likely limited the activity of photosynthetic pathways but did not hinder artemisinin accumulation. This suggests that genetic transformation compensates for the absence of light stimulation by inducing transgene expression that regulates secondary metabolism. Compared with *A. annua*, the results for *A. vulgaris* were less pronounced. The low artemisinin content (0.28 ± 0.03 mg/g) and slow biomass growth (9.8 ± 0.9 g/L) may be attributed to the absence of specific regulatory elements analogous to TAR2 or AaWRKY9 in this species. R. Judd *et al*. (2023) demonstrated that metabolic engineering of the anthocyanin pathway in *A. annua* can influence artemisinin biosynthesis through competitive interactions. In the case of *A. vulgaris*, similar mechanisms are likely to be less effective, highlighting the need for species-specific approaches to genetic modification.

The findings of this study reveal substantial differences in the efficiency of genetic transformation and the accumulation of bioactive

compounds between *Artemisia annua* and *Artemisia vulgaris*. The high transformation rate and enhanced bioactivity of *A. annua* roots confirm the potential of this species for pharmaceutical applications, whereas the results for *A. vulgaris* indicate the need for further research and refinement of transformation strategies.

CONCLUSIONS

The results of the study demonstrate the high efficiency of genetic transformation in *Artemisia annua* and *A. vulgaris* using *Agrobacterium rhizogenes*, as evidenced by the frequency of transgenic root formation (78.3% for *A. annua* and 65.0% for *A. vulgaris*). The differences between the species are attributed to varying tissue sensitivity to infection, structural characteristics of the cell wall, and the efficiency of T-DNA integration into the genome. *A. annua* exhibited higher expression of receptors (FLS2, EFR) and activity of proteins (*VIP1*), which facilitated more rapid T-DNA transport to the nucleus and more stable expression of the *rolB/C* gene. Morphologically, *A. annua* transgenic roots displayed more intense branching and faster growth, reaching a maximum biomass of 12.4 ± 1.2 g/L by day 28, which was 1.8 times higher than that of *A. vulgaris* (9.8 ± 0.9 g/L by day 35).

The elevated levels of bioactive compounds in the transgenic roots of *A. annua* (artemisinin – 1.45 ± 0.15 mg/g, flavonoids – 25.6 ± 2.1 mg QE/g, sugars – 12.3 ± 1.0 mg/g) compared with the control groups (artemisinin – 0.45 ± 0.05 mg/g, flavonoids – 8.2 ± 0.9 mg QE/g, sugars – 4.2 ± 0.5 mg/g) confirm the effectiveness of the transformation in stimulating secondary metabolism and highlight the potential of genetic modification to enhance the bioactivity of this species. A strong correlation between flavonoid content and antioxidant activity (r = 0.89), as well as a moderate correlation between sugar content and antiviral activity (r = 0.76), indicates a multifaceted mechanism of biological activity. Transgenic extracts of A. annua inhibited the replication of the influenza A/H1N1 virus by 68% ± 5%, exceeding the inhibition observed in control samples by 26%, thus demonstrating the potential for the development of antiviral agents.

Despite the success achieved with *A. annua*, the low artemisinin content in *A. vulgaris* (0.28 ± 0.03 mg/g) and slower biomass growth highlight the need to optimise cultivation conditions for this species. Promising strategies include adjusting hormonal balance – particularly the ratio of auxins to cytokinins to stimulate root development – increasing sucrose concentration in the medium to enhance cellular energy supply, and employing *A. rhizogenes* strains with elevated *vir* gene activity to improve transformation efficiency and T-DNA delivery.

The data confirm that transgenic roots of *A. annua* hold promise for the biotechnological production of artemisinin, antioxidants, and antiviral compounds. However, further *in vivo* studies are required for clinical application, particularly to evaluate toxicity, pharmacokinetics, and efficacy under physiologically relevant conditions. This study establishes a foundation for the pharmaceutical and biotechnological use of *Artemisia*, emphasising the importance of species-specific approaches in genetic transformation. Future research could explore the introduction of root-specific transcription factors and examine in greater detail the role of hormonal signalling in the biosynthesis of active compounds. Nonetheless, the current study is limited by the inclusion of only two *Artemisia* species, and subsequent work should incorporate a broader range of species to validate the overall effectiveness of the proposed methods.

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Отримання культури трансгенних коренів рослин *Artemisia annua* L., *Artemisia vulgaris* L., визначення вмісту біологічно активних сполук (артемізину, флавоноїдів та цукрів) і біологічної активності (антиоксидантної та противірусної) в отриманих коренях

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Анотація. Метою дослідження було оцінити ефективність генетичної трансформації коренів *Artemisia annua* та *Artemisia vulgaris* за допомогою бактерії *Agrobacterium rhizogenes* та визначити вплив цього процесу на вміст біологічно активних сполук і їхню біологічну активність. Експеримент, проведений в умовах *in vitro*, включав інфікування молодих експлантів штаммами *A. rhizogenes* ATCC 15834 та A4 з подальшим культивуванням трансгенних коренів у рідкому середовищі Мурасіге-Скуга. Концентрація флавоноїдів визначалась спектрофотометрично за реакцією з хлоридом алюмінію, антиоксидантну властивість визначали за допомогою тестів зі стабільними радікалами DPPH та ABTS. Ефективність трансформації склала 78,3 ± 4,2 % для *Artemisia annua* та 65,0 ± 5,1 % для *Artemisia vulgaris*, що пов'язано з відмінностями у структурі клітинної стінки та експресії рецепторів, таких як FLS2 і EFR. Вміст артемізину в трансгенних коренях *Artemisia annua* досяг 1,45 ± 0,15 мг на грам сухої маси, що в 3,2 рази перевищує контрольні значення (0,45 ± 0,05 мг/г), тоді як у *Artemisia vulgaris* цей показник становив лише 0,28 ± 0,03 мг/г. Концентрація флавоноїдів склала 25,6 ± 2,1 мг еквівалентів кверцетину на грам для *Artemisia annua* та 18,9 ± 1,7 мг еквівалентів кверцетину на грам для *Artemisia vulgaris*. Антиоксидантна активність показала, що половина максимальної інгібуючої концентрації для *Artemisia annua* становила 32,5 ± 2,8 мкг/мл у DPPH-тесті, що на 45 % нижче за контроль. Екстракти *Artemisia annua* демонстрували противірусну активність, інгібуючи реплікацію вірусу грипу A/H1N1 на 68 ± 5 %, тоді як для *Artemisia vulgaris* цей показник склав 55 ± 4 %. Статистичний аналіз підтвердив значущість відмінностей між видами ($p < 0,05$). Отримані дані створюють основу для розробки більш ефективних препаратів на основі трансгенних коренів *Artemisia annua*, зокрема протималярійних засобів із підвищеним вмістом артемізину, а також антиоксидантних та противірусних агентів для профілактики та лікування інфекційних захворювань

Ключові слова: генетична трансформація; *Agrobacterium rhizogenes*; антималярійний агент; фармацевтичні препарати; протизапальні властивості