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Analysis of Total Phenolic Contents in the Bark of Different Mulberry Varieties (*Morus* sp.)

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ABSTRACT

Mulberry (*Morus* sp.) trees are renowned for their rich profile of bioactive compounds, particularly phenolics, which vary among tissues and genotypes. This study investigated the total phenol contents in the bark of one-year-old shoots of different mulberry species and genotypes. In addition, the article compares total phenolic contents between bark and leaves. The study included reference sericultural cultivars of *M. alba* and *M. indica*, two local Slovenian white mulberry genotypes, and a hybrid between white and red mulberry (*M. alba* × *M. rubra*). One-year-old shoots were sampled during the winter dormancy period, after which the bark was peeled, lyophilised, and ground into a fine powder. The total phenolic contents were analysed using the Folin-Ciocalteu method. The results showed significant differences in total phenolic content among species and genotypes.The highest content was observed in the *M. alba* × *M. rubra* hybrid [5.14 ± 0.01 mg gallic acid equivalent (GAE) g^{-1} dry weight (DW)], and the lowest in the sericultural cultivar 'Kokusou' (4.60 ± 0.08 mg GAE/g^{-1} DW). The comparison between bark and leaves showed that total phenolic concentrations were lower in the bark than in the leaves. The results indicate that both genetic background and plant tissue type influence phenolic compound levels, underscoring the need to consider these factors in future studies on bioactive compounds in mulberries.

Keywords: mulberry shoots, chemotypic differences, secondary phloem, leaves, bioactive compounds

INTRODUCTION

Mulberries (*Morus* sp.) are perennial deciduous trees belonging to the family Moraceae, adapted to tropical, subtropical, and temperate regions, and are therefore distributed almost worldwide (Plants of the World Online, 2026). Mulberries have been valued since ancient times for their diverse uses, cultivated not only as ornamental and fruit-bearing trees but also for livestock feed and high-quality timber. Recent review studies have highlighted the medicinal potential of several mulberry organs, including leaves, fruits and bark, which contain bioactive compounds associated with antioxidant, anti-inflammatory, antidiabetic, antimicrobial, neuroprotective, and cardiometabolic effects (Zafar et al. 2013, Dadhwal and Banerjee, 2023; Liu et al., 2023; Fatima et al. 2024, James et al., 2024; Wang et al., 2024; Li et al., 2025; Yan et al., 2025).

Studies focusing on the phytochemical composition and pharmacological properties of mulberry bark are limited; however, recent research has shown that bark contains bioactive compounds with significant anti-inflammatory potential (Senekovič et al. 2025).

Recent studies indicate that bioactive compounds from *M. alba* shoots, particularly polysaccharides, alkaloids, and stilbenes, exhibit significant hypoglycemic activity through the regulation of glucose and lipid metabolism, enhancement of insulin sensitivity, and protection of pancreatic tissue from oxidative stress, supporting their potential use in the management of diabetes mellitus (Liu et al., 2023).

Phenols are aromatic alcohols containing one or more hydroxyl groups attached to a benzene ring, and they belong to the group of secondary metabolites. These are metabolic compounds of plants, fungi, or lichens, derived from primary metabolites (sugars, amino acids, fatty acids), with various functions such as defense, signaling, inhibition, or toxicity. They are generally not directly involved in processes of growth and development (Batič et al., 2011). Plant phenolics include phenolic acids, flavonoids and tannins, as well as the less common stilbenes and lignans. Flavonoids represent the most abundant class of polyphenols in the human diet (Dai and Mumper, 2010).

Wu et al. (2022) employed untargeted metabolomics to investigate the metabolites in leaves and shoot bark of white

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mulberry (*M. alba*), revealing marked differences in metabolite composition and antioxidant activity between plant tissues as well as male and female individuals. The study demonstrated that female individuals exhibited higher antioxidant activity compared to males, providing an important basis for future research on sex-related differences in dioecious plants and their potential applications in various fields.

Memete et al. (2022) characterised the phytochemical profile of different mulberry organs, with emphasis on phenolic compounds and their health benefits. Flavonoids were identified as the dominant metabolites, abundant in leaf epidermis, fruit exocarp, one-year-old shoot and root bark, with prenylated flavonoids such as morusin being particularly abundant. Morusin is widely distributed in vegetative tissues and is considered one of the key phenolic compounds of mulberry, which has attracted attention due to its reported biological activities. Panek-Krzyśko and Stompor-Gorący (2021) reported that morusin exhibits anticancer, antimicrobial, anti-inflammatory, and neuroprotective effects, although its practical application is limited by low bioavailability.

Chang et al. (2011) investigated the antioxidant and antityrosinase activity of one-year-old shoots and root bark of *M. alba*. In ethanolic extracts of shoot bark, they reported a total polyphenol content of 60.25 mg GAE/g DW. Using high-performance liquid chromatography (HPLC), they identified five bioactive phenolic compounds, including maclurin, rutin, isoquercitrin, and morin, which were present at higher levels in shoot bark compared to root bark.

Thabti et al. (2014) evaluated the antioxidant and antimicrobial activity of leaves and shoot bark from three mulberry taxa (*M. alba* var. *alba*, *M. alba* var. *rosa*, and *M. rubra*) to assess their therapeutic potential. Total phenolic and flavonoid contents were measured in hydromethanolic and aqueous extracts. Highest levels of total phenols in shoot bark were found in *M. alba* var. *alba*. Aqueous extracts showed greater concentrations compared to hydromethanolic extracts, confirmingthat aqueous extracts of mulberry leaves and bark represent a rich source of polyphenols with antioxidant and antimicrobial properties.

The aim of this study was to investigate the total phenolic content in the bark of one-year-old mulberry shoots of different genotypes. Special emphasis was given to the comparison between the bark of the hybrid *M. alba × M. rubra*, the reference sericultural cultivars and the local genotypes of white mulberry. In addition, we compared the total phenolic content between the bark and the leaves of mulberry genotypes. The study was designed to test whether total phenolic content in bark differs among mulberry genotypes and species, and whether total phenolic content is lower in bark than in leaves.

MATERIALS AND METHODS

Plant material

The bark of one-year-old mulberry shoots of three different genotypes were used in our study. Alltogether, we analysed 14 different mulberry genotypes, including 11 reference sericultural cultivars ('Florio', 'Giazzola', 'Kokusou', 'Morettiana') two local Slovenian white mulberry genotypes (P 137.1, P 137.15), and one hybrid between a white and a red mulberry (*M. alba × M. rubra*). Samples from several trees of the same cultivar (N=3) were used in the analyses. Unfortunately, this approach was not possible for all genotypes because the number of available trees was limited. In the case of the *M. alba × M. rubra* hybrids, the individual trees represented different genetic backgrounds; therefore, the hybrids were analysed on an individual basis. Sampling of one-year-old mulberry shoots for the analysis of total phenols in bark of different species and genotypes was carried out during winter dormancy on 4th of December 2023 and 4th of January 2024 in the mulberry collection orchard under Villa Pohorski dvor Slovenia (ϕ: 46° 50' 88.33"; λ: 15° 62' 20.73"). Biochemical analyses of the samples were performed in the laboratories of the Faculty of Agriculture and Life Sciences. One-year-old shoots were collected from the tested mulberry genotypes and transported in water to the Faculty greenhouse. Bark was peeled from the first five nodes using a scalpel, excluding buds, and immediately packed in paper bags. Samples were stored in liquid nitrogen and subsequently transferred to a −80 °C freezer. Bark was then lyophilised for seven days at −50 °C under 0.08 mbar. After lyophilisation, bark samples were cut into small pieces, pre-softened in a mortar, and ground in a dismembrator (Tissuelyser II) using 10 mL Teflon adapters with a 20 mm ball for 4 min at 20 Hz. The resulting material was sieved to obtain a fine powder, which was stored in airtight vials at −20 °C until biochemical analysis. The concentration of total phenols is calculated on a dry weight (DW) basis.

Extract preparation for total phenolic analysis

For the analyses of total phenolic compounds 150 ± 2 mg of ground, lyophilised material was weighted in duplicate for each sample and homogenised with 3 mL of 3% formic acid in 70% methanol. Throughout the analytical process, the samples were kept in an ice bath due to the sensitivity of the bioactive compounds. The samples were then incubated for 60 minutes in an ultrasonic bath with ice insert. Samples were centrifuged at 4 °C for 15 min at 2800 rpm. We then transfered 100 µL of the supernatant to new microcentrifuges which we protected from sunlight. Next 200 µL of freshly prepared Folin's reagent [1 mL Folin's

reagent and 9 mL ddH_2O] was added. Another 800 µL Na_2CO_3 solution [18.5 g Na_2CO_3 with 200 mL ddH_2O] was added. The samples were then shaken in the dark at 30 rpm for two hours at room temperature.

Determination of the total phenolic content

Total phenolic contents were quantified using the Folin-Ciocalteu method following the protocol decribed by Ainsworth and Gillespie (2007). Absorbance was measured at 765 nm using a Varian Cary 50 Bio spectrophotometer. The spectrophotometer was calibrated with a blank solution, the gallic acid standards were measured, and finally the extracts were analysed. The calibration curve was prepared using gallic acid standards in the concentration range of 0.052–0.280 mg/mL. Total phenol content was quantified using the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW). Absorbance values were converted to phenolic concentrations based on the calibration range and subsequently normalized to sample dry weight, with results reported as mg g^{-1} GAE per DW (e.g., 5.14 ± 0.01 mg g^{-1} DW).

Statistical analyses

Each genotype was analysed in two to three independent replicates (N=2-3), and all samples were measured in duplicate. Analytical data (date of analysis, sample name, sample weight, and absorbance values) were recorded and processed for statistical evaluation using IBM SPSS Statistics (Version 29). Sample concentrations (based on the standard calibration curve), means, and standard deviations were calculated. Since the variables did not meet the assumptions for parametric tests, non-parametric methods were applied. Differences in total phenol content among genotypes were assessed using the Kruskal–Wallis test for independent samples, followed by a post-hoc analysis with Bonferroni correction to group genotypes into homogeneous subsets. The Kruskal–Wallis test was also used to evaluate differences between species. Statistical significance was assessed at $\alpha = 0.05$, with p-values < 0.05 considered statistically significant.

RESULTS

Total phenolic content in bark among mulberry genotypes

Total phenolic contents were determined in the bark of one-year-old shoots of historical Slovenian white mulberry trees, reference sericultural cultivars, and one hybrid genotype. The median total phenolic content across all analysed genotypes was 4.97 ± 0.19 mg GAE/g DW. The lowest value was recorded in the sericultural cultivar 'Kokusou' (4.60 ± 0.08 mg GAE/g DW), whereas the highest value was determined in the *M. alba* × *M. rubra* hybrid (5.14 ± 0.01 mg GAE/g DW) (Figure 1). These findings confirm statistically significant variation in total phenolic contents among genotypes (p = 0.001, $\alpha = 0.05$).

Comparison between bark and leaves

Total phenolic content in bark was also compared with previously determined values for leaves of the same mulberry genotypes. In bark, the total phenolic content ranged from 4.60 ± 0.08 to 5.14 ± 0.01 mg GAE/g DW. In contrast, the median total phenolic content in leaves was substantially higher, reaching 20.14 ± 1.57 mg GAE/g DW (Table 1). The lowest value in leaves was recorded in the Slovenian local genotype P 137.15 (15.97 ± 0.44 mg GAE/g DW), while the highest value was observed in the reference sericultural cultivar 'Kokusou' (21.36 ± 0.02 mg GAE/g DW). Overall, the results indicate lower phenolic content in bark than in leaves across the analysed mulberry material (Table 1).

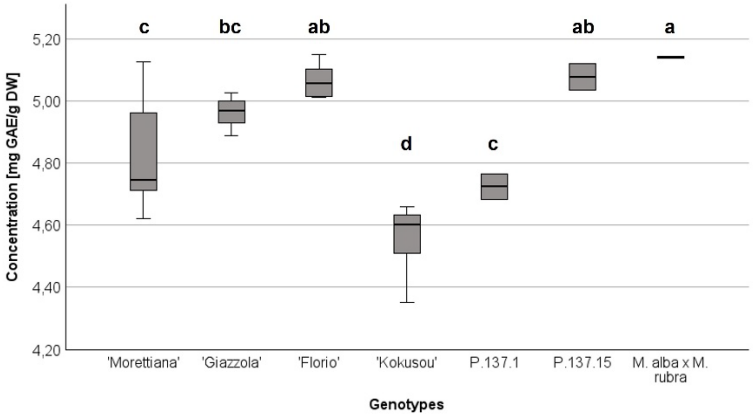

Figure 1: Mean, minimal an maximal values of total phenol contents in analysed genotypes (N=2-3). Different letters (a, b, c, d) indicate significant differences (p < 0.05) among the examined genotypes

Table 1: Comparison of total phenolic content (median ± median deviation) between bark and leaves of reference sericultural cultivars, Slovenian local genotypes, and the hybrid *M. alba* × *M. rubra* [mg GAE/g DW]

Genotype	Total phenols in bark [mg g^{-1} DW]	Total phenols in leaves [mg g^{-1} DW]
Reference sericultural cultivars		
'Morettiana'	4.75 ± 0.13 c	18.75 ± 0.06
'Giazzola'	4.97 ± 0.04 bc	19.15 ± 0.79
'Florio'	5.06 ± 0.04 ab	20.54 ± 0.32
'Kokusou'	4.60 ± 0.08 d	21.36 ± 0.02
Slovenian local genotypes		
P 137.1	4.72 ± 0.04 c	–
P 137.15	5.08 ± 0.04 ab	15.97 ± 0.44
Hybrid		
M. alba × *M. rubra*	5.14 ± 0.01 a	20.77 ± 1.20

DISCUSSION

The present study demonstrated that total phenolic content in the bark of one-year-old mulberry shoots differs significantly among genotypes. This confirms that the observed variations are associated with genetic background, but they also reflect differences in plant physiological status, environmental conditions, and tolerance to environmental stress factors. Phenolic compounds are well known to respond to external factors such as drought, ultraviolet radiation, or pathogen pressure, which may additionally contribute to inter-genotypic variation (Kumar et al., 2023; Saini et al., 2024; Nurzyńska-Wierdak, 2023; Xu and Wang, 2025).

The phenolic values obtained in this study were somewhat higher than those reported by Thabti et al. (2014), who found 2.54–3.04 mg GAE/g DW in the bark of white and red mulberry shoots. Such differences may be related not only to genotype and growing conditions, but also to differences in plant age, sampling strategy, and analytical procedures. By contrast, Khan et al. (2013) reported markedly higher values (285.62 ± 2.54 mg GAE/g DW), which most likely reflect major differences in extraction methodology. In that study, the bark was air-dried, macerated in methanol for 15 days, and the extract was concentrated under reduced pressure before phenolic analysis. Similarly, Chang et al. (2011) reported a high polyphenol content (60.25 mg GAE/g DW) in the bark of white mulberry shoots, which may also be attributed to methodological differences, including ethanol extraction, rotary evaporation, and analysis of concentrated dry extracts. These comparisons underline the importance of extraction and sample preparation protocols when interpreting total phenolic values across studies.

A particularly interesting result was the lowest total phenolic content observed in the bark of the cultivar 'Kokusou', which coincided with the highest phenolic content in its leaves. The variety 'Kokusou' was introduced by Japanese breeders in the 1920s as *M. latifolia* (Cappellozza, 2002), although its precise taxonomic placement remains debated. Traditionally, var. *latifolia* 'Kokusou' has been associated with *M. alba* (Cappellozza, 2002; Machii et al., 2002), whereas current taxonomic sources, such as POWO (2026) and World Flora Online (2026), place it within the broader taxonomic framework of *M. indica*. This taxonomic ambiguity may therefore be relevant when interpreting biochemical variation among mulberry genotypes.

The comparison between species further showed that the *M. alba* × *M. rubra* hybrid had significantly higher total phenolic content than the analysed *M. alba* genotypes. This finding differs from the observations of Thabti et al. (2014), who reported the highest phenolic content in *M. alba* bark and the lowest in *M. rubra*. In the present study, however, the hybrid bark showed the highest value among all analysed material, suggesting that the hybrid genetic background may influence the accumulation of phenolic compounds. This may reflect additive or non-additive effects of parental genomes on secondary metabolism, although this interpretation would require confirmation in a broader set of hybrid genotypes.

When shoot colour is taken into account, clear differences can be observed among the analysed genotypes. 'Kokusou' is characterised by greyish-brown shoots, P136.11 and P136.15 by greenish-brown shoots, 'Morettiana' and *M. alba* × *M. rubra* by reddish-brown shoots, and 'Giazzolla' by dark-brown shoots, as shown in Figure 2. These differences suggest that bark or shoot pigmentation may be related to phenolic accumulation, with darker bark potentially associated with higher phenolic content. However, the physiological relationship between lower phenolic content in the bark and higher phenolic content in the leaves, should be investigated further in a broader analysis, ideally including molecular-level studies of phenolic biosynthesis and allocation among plant tissues.

The comparison between bark and leaves clearly indicated that phenolic concentrations were lower in bark than in leaves. This pattern is in agreement with Thabti et al. (2014), who also found higher phenolic content in leaves than in shoot bark. Higher phenolic accumulation in leaves is physiologically plausible, as leaves are metabolically more

active tissues and represent a primary site of interaction with environmental stressors such as light, herbivory, and pathogens. In this context, a higher concentration of phenolics may contribute to antioxidant defence and protective functions. The lower concentrations found in bark may reflect its distinct structural and physiological role.

Nevertheless, the literature does not show a fully consistent pattern. Zou et al. (2012) reported higher total phenolic contents in mulberry leaves than those observed by Jelen (2022), most likely due to methodological differences, particularly the use of 70% ethanol extraction and concentration of extracts prior to analysis. In contrast, Khan et al. (2013) found substantially higher phenolic content in bark than in leaves of *M. alba*, with bark values approximately 2.75 times higher than leaf values. This discrepancy again highlights the strong influence of extraction method, solvent system, and sample processing on reported total phenolic contents.

Overall, the present results show that both genotype and plant tissue type influence total phenolic content in mulberry. The findings support the view that bark and leaves should be considered separately in studies of mulberry bioactive compounds, and that careful methodological standardisation is necessary for meaningful comparison among genotypes, tissues, and studies. Further research including a larger number of genotypes, additional hybrid material, and harmonised analytical procedures would help to clarify the biological and taxonomic patterns underlying phenolic variation in mulberry.

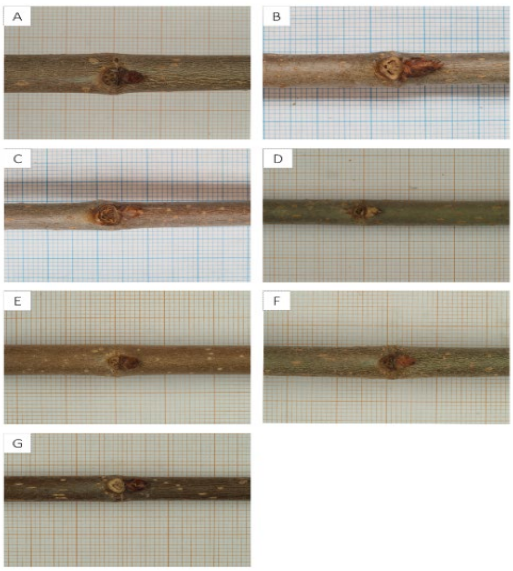

Figure 2: Colour of the one-year old shoots of analysed genotypes. A 'Morettiana'; B 'Giazzola'; C 'Florio'; D 'Kokusou'; E P137.1; F P137.15; G *M. alba* × *M. rubra*. For further detailes on the morphological traits of the analysed genotypes see the Catalogue of Mulberry Varieties https://aracneproject.eu/catalogue-of-the-mulberry-varieties/

CONCLUSIONS

The present study revealed significant variation in total phenol content among mulberry genotypes, with the hybrid *M. alba* × *M. rubra* exhibiting the highest levels and the sericultural cultivar 'Kokusou' the lowest. Differences were also confirmed at the species level, as phenol content in the hybrid was significantly higher than in *M. alba* and *M. indica* genotypes. Furthermore, a comparison with previous data on leaves of the same genotypes demonstrated that phenolic content is markedly higher in leaves than in bark, likely reflecting differences in physiological function between tissues. These results highlight the strong influence of genetic background, species, and tissue type on phenolic composition in mulberries. They also emphasise the need for further research including larger sample sets, diverse environmental conditions, and complementary analytical approaches to better understand the distribution and functional role of phenolic compounds in mulberry tissues. In the future, it would also be useful to analyse tannins in mulberry bark to complement the findings on total phenols.

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Analiza vsebnosti skupnih fenolov v skorji različnih genotipov murv (*Morus* sp.)

IZVLEČEK

Murve (*Morus* sp.) so znane po bogatem profilu bioaktivnih spojin, zlasti fenolnih spojin, katerih vsebnost se razlikuje med rastlinskimi tkivi in genotipi. V tej študiji smo proučevali vsebnost skupnih fenolov v skorji enoletnih poganjkov različnih vrst in genotipov murv. Poleg tega članek primerja vsebnost skupnih fenolov med skorjo in listi. Študija je vključevala referenčne svilogojske kultivarje vrst *M. alba* in *M. indica*, dva lokalna slovenska genotipa bele murve ter križanca med belo in rdečo murvo (*M. alba* × *M. rubra*). Enoletni poganjki so bili vzorčeni v obdobju zimskega mirovanja, nato je bila skorja olupljena, liofilizirana in zmleta v fin prah. Vsebnost skupnih fenolov je bila določena z metodo Folin-Ciocalteu. Rezultati so pokazali značilne razlike v vsebnosti skupnih fenolov med vrstami in genotipi. Najvišja vsebnost je bila ugotovljena pri križancu *M. alba* × *M. rubra* [5,14 ± 0,01 mg ekvivalenta galne kisline (GAE) g^{-1} suhe mase (DW)], najnižja pa pri svilogojskem kultivarju 'Kokusou' (4,60 ± 0,08 mg GAE g^{-1} DW). Primerjava med skorjo in listi je pokazala, da so bile koncentracije skupnih fenolov v skorji nižje kot v listih. Rezultati kažejo, da tako genetsko ozadje kot tip rastlinskega tkiva vplivata na ravni fenolnih spojin, kar poudarja potrebo po upoštevanju teh dejavnikov v prihodnjih raziskavah bioaktivnih spojin pri murvah.

Ključne besede: poganjki murve, kemotipske razlike, sekundarni floem, listi, bioaktivne spojine