ORIGINAL PAPER

CHARACTERISATION OF NUTRACEUTICAL FATTY ACIDS AND AMINO ACIDS IN THE BUDS OF *POPULUS NIGRA*, *POPULUS ALBA*, AND *POPULUS X EURAMERICANA* BY GAS CHROMATOGRAPHY

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Abstract. Populus species (poplars) are deciduous trees commonly found in the Northern Hemisphere, typically growing along rivers, in meadows, and urban environments. Their buds, leaves, and bark contain important bioactive compounds with known anti-inflammatory, antiseptic, and analgesic properties. In this study, the buds of three Populus species (P. nigra, P. alba, and P. × euramericana) were analyzed for their bioactive compounds, focusing on fatty acid and amino acid profiles. Ethanolic extracts were evaluated using gas chromatography-mass spectrometry (GC-MS), revealing a diverse composition of nutraceutical compounds. Key fatty acids such as oleic acid, linoleic acid, and α-linolenic acid, as well as glycerides like 1-monopalmitin and glyceryl monostearate, were identified. Essential amino acids, including L-alanine, L-valine, and L-proline, were also detected. The results indicated a high content of unsaturated fatty acids and amino acids, supporting the potential use of poplar bud extracts as valuable sources of nutraceuticals and bioactive ingredients for applications in health and cosmetology.

Keywords: Populus, fatty acids, amino acids, GC-MS, nutraceuticals, bioactive compounds

1. INTRODUCTION

Populus trees, belonging to the Salicaceae family, are commonly found in the southern regions of Romania, growing wild in plains, low hills, near watercourses, or even in urban areas. These species are characterized by columnar crowns and simple, typically triangular leaves. Their unisexual flowers, arranged in long, pendulous catkins without a calyx or corolla, bloom in early spring from buds, before the leaves emerge [1]. The most common species of Populus species growing in the Northern and Central Europe are: Populus nigra L. (black poplar), Populus alba L. (white poplar), Populus tremula L. (European aspen), and the hybrid Populus x euroamericana (Dode) Guinier (Euroamerican poplar).

In Romania, white poplar is found in plains and low hills, particularly in river meadows, starting from the Danube Delta, forming pure or mixed stands with black poplar, willows, or black alder [1,2].

Black poplar grows in wet meadows and depressions, as well as in the valleys of plains and hills. Its distribution extends to higher altitudes compared to white poplar. It is also cultivated as an ornamental tree and is often planted along roadsides. The most common

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varieties of black poplar in Romania include *Populus nigra* L. cv. *Italica*, *P. nigra* L. cv. *Thevestina*, and *P. nigra* var. *plantierensis* (Simon-Louis) Schneid. [1,2].

Populus x euramericana Moench (P. x canadensis) (Dode) Guinier is a hybrid tree resulting from the crossbreeding of the European black poplar with the American black poplars such as P. deltoides Marsh, native to Canada and the north of the USA, or P. angulata Ait., found in central and north of North America. The main cultivars currently allowed in cultivation in Romania, consist in: cv. Serotina (clones R1, R3, R4), cv. Robusta (clones R13, R18, R20, Oltenita, and especially clone R16), cv. Sacrau (clones 59 and 79), cv. Italy (clones I214, I45/51, I45/55, I69/55), cv. Deltoides 227, cv. Regenerated, cv. Citadel, cv. Arges, cv. Marilandica, cv. Jacometti (clones 107, 307), cv. Toropogritski. [2]. The large-scale cultivation of hybrid black poplars has been widely practiced in Romania, particularly in the inner river meadows, the plains, and especially in the Danube Meadow, with additional plantations in the Danube Delta. Plantations also exist in the Danube Delta. The area of these cultures is currently about 51,000 ha, of which approximately 22,000 ha is in the Danube Meadow, 6,000 ha in the Danube Delta (in total in the Danube Meadow and Delta 28,000 ha), and approximately 23,000 ha in the inland river meadows [1,2].

Populus species growing in Romania contain in their buds, leaves, or bark different classes of chemical compounds, such as phenolic acids, flavonoids, tannin, phenolic glycosides, essential oil, saponins, resins, vanillin, sugars few alkaloids [3-5]. Due to the presence of these important active ingredients, poplar is widely used for its anti-inflammatory, antipyretic, analgesic, and antiseptic properties, and many other biological effects (cytotoxic on different cancer cell lines, antioxidant, antibacterial, antifungal) have been recently discovered [3-6].

Nutritional therapy and phytotherapy have gained significant importance in recent years as people seek natural approaches to health and wellness. The widespread adoption of these approaches, which emphasize the consumption of nutraceuticals, natural plant-based foods, and other dietary interventions, has become increasingly popular [7-12]. These methods are often recommended for enhancing overall health, as well as preventing and managing various diseases. With growing awareness of the benefits of natural treatments and a shift towards more sustainable and preventive healthcare, these healing systems are becoming increasingly integrated into mainstream health practices. Among the nutraceutical compounds, fatty acids and amino acids play an important role for human nutrition and health. It is known that fatty acids are major components of cell membranes, being critical for brain development and function, and amino acids are key elements for the synthesis of proteins, enzymes, and hormones, both providing a source of energy and having many biological actions [12].

This study aims to discover important nutraceutical fatty acids and amino acids in the buds of three species of poplar growing wild in the South of Romania, by a detailed gas-chromatography analysis, coupled with mass spectrometry, as an efficient, potential source of nutraceuticals and active ingredients that can be used in cosmetics [13,14].

2. MATERIALS AND METHODS

2.1. MATERIALS

Male floral buds from three *Populus* species growing in Southern Romania were collected in March 2024 from Oinacu Village, Giurgiu County. The three species were

Populus nigra L. (black poplar), *Populus alba* L. (white poplar), and the interspecific hybrid *Populus* × *euramericana* (Dode) Guinier (Euramerican poplar). All plant specimens were identified at the Botany Laboratory of Titu Maiorescu University, Faculty of Pharmacy.

The plant material was dried at room temperature in the shade and then ground. Ethanolic extracts were prepared using 1 g of dried plant material with 100 mL of 50% ethanol by refluxing for 30 minutes at 100°C in a water bath. The solutions were then filtered and brought to volume with the solvent in a 100 mL graduated flask.

2.2. METHODS

2.2.1. Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds: Profiling of Nutraceutical Amino Acids and Fatty Acids in Poplar Extracts

Gas chromatography coupled with mass spectrometry (GC-MS) was employed to analyze the presence of fatty acids and amino acids in the bud extracts of three *Populus* species. The analysis was carried out using a TRACE 1310 GC system (Thermo Scientific, Waltham, MA, USA), which included a TriPlus TSH autosampler and a TSQ EVO 8000 triple quadrupole mass spectrometer, operated via Chromeleon software.

For sample preparation, 50 µL of ethanolic extract was evaporated to dryness under a nitrogen stream. The dry residue was then derivatized using N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA). Chromatographic separation was achieved on an HP-5MS column (30 m), under the following conditions: carrier gas flow rate of 1 mL/min, injection volume of 1 µL, injector temperature of 280°C, and a split ratio of 60:1. The oven temperature was programmed to increase from 100°C to 280°C at a rate of 10°C/min. Identification of compounds was performed by comparing the retention times and mass spectra with those of a commercial standard mixture (Mix 275 ME) and reference data from the NIST MS library, version 2.2. Using GC-MS analysis, it was identified the components of interest and their relative proportions within the overall chemical profile were identified. The absolute quantification of fatty acids in extracts was performed using calibration curves and internal standards.

2.2.2. Fatty Acid Assay

To perform the fatty acid assay, 0.50 g of ethanolic extract from the buds of three *Populus* species was weighed into a 20 mL volumetric flask. Next, 5 mL of hexane, 0.5 mL of 0.5 M methanolic sodium hydroxide, and 5 mL of methanol were added. The flask was sealed and heated in an oven at 80 °C for 30 minutes.

After cooling to room temperature, 0.5~mL of 14% boron trifluoride (BF₃) solution was added, and the flask was reheated at 80°C for an additional 10 minutes. Following a second cooling step, 2~mL of saturated sodium chloride solution was added to facilitate phase separation. The contents were mixed by inversion, and the upper organic phase (hexane) was collected and filtered through a $0.2~\mu\text{m}$ PTFE membrane filter.

The filtered extract was analyzed using a GC-MS/MS system (Trace 1310/TSQ 8000 Evo) equipped with a TG-5 SILMS column (30 m \times 0.25 mm \times 0.25 μ m). Chromatographic conditions included a split injector at 250°C (split ratio 1:80), an injection volume of 2 μ L,

and an oven temperature program starting at 170°C (held for 2 min), followed by two temperature ramps up to 260°C. Additional parameters included a transfer line temperature of 280°C and an ionization source temperature of 230°C [15].

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Gas Chromatography-Mass Spectrometry Analysis of Bioactive Compounds: Nutraceuticals, Amino Acids, and Fatty Acids Profiling

The 50% ethanolic extracts obtained from the buds of the three *Populus* species revealed a diverse profile of bioactive compounds, primarily comprising amino acids and fatty acids. The identification was based on GC-MS analysis of the silylated derivatives, whose retention times are detailed in Table 1.

Table 1. Fatty acids and amino acids identified by GC-MS in the buds of *Populus* species.

Peak Name	Phytochemical Class	Formula	Retention	Relative		
			Time [min]	Area [%]		
Populus nigra L. L-Alanine, 2TMS Amino acids C ₀ H ₂₃ NO ₂ Si ₂ 5.62 0.52						
L-Alanine, 2TMS	Amino acids	C ₉ H ₂₃ NO ₂ Si ₂		0.52		
L-Valine, 2TMS	Amino acids	$C_{11}H_{27}NO_2Si_2$	7.71	0.31		
L-Proline, 2TMS	Amino acids	$C_{11}H_{25}NO_2Si_2$	9.20	0.44		
Palmitic acid, TMS	Fatty acids	$C_{19}H_{40}O_2Si$	22.75	2.63		
Linoleic acid, TMS	Fatty acids	$C_{21}H_{38}O_2Si$	24.78	4.43		
Oleic acid, TMS + α -Linolenic acid	Fatty acids	$C_{21}H_{38}O_2Si$	24.84	11.44		
Stearic acid, TMS	Fatty acids	$C_{21}H_{44}O_2Si$	25.13	0.88		
Eicosenoic acid, TMS	Fatty acids	$C_{23}H_{48}O_2Si$	26.87	3.92		
1-Monopalmitin, 2TMS	Fatty acid glycerides	$C_{25}H_{54}O_4Si_2$	28.58	10.93		
Glyceryl monostearate, 2TMS	Fatty acid glycerides	$C_{27}H_{58}O_4Si_2$	31.11	5.45		
Populus alba L.						
L-Alanine, 2TMS	Amino acids	C ₉ H ₂₃ NO ₂ Si ₂	5.63	0.60		
L-Valine, 2TMS	Amino acids	$C_{11}H_{27}NO_2Si_2$	7.71	0.43		
L-Proline, 2TMS	Amino acids	$C_{11}H_{25}NO_2Si_2$	9.21	2.75		
Serine, 2TMS	Amino acids	C ₉ H ₂₃ NO ₃ Si ₂	10.18	0.93		
L-Threonine, 3TMS	Amino acids	C ₁₃ H ₃₃ NO ₃ Si ₃	10.60	0.35		
L-5-Oxoproline, 2TMS	Amino acids derivate	$C_{11}H_{23}NO_3Si_2$	24.85	12.38		
Linoleic acid, TMS	Fatty acids	$C_{21}H_{38}O_2Si$	24.78	3.98		
Oleic acid + α-Linolenic acid	Fatty acids	$C_{21}H_{38}O_2Si$	24.85	12.38		
Stearic acid, TMS	Fatty acids	$C_{21}H_{44}O_2Si$	25.12	1.35		
11-Eicosenoic acid, TMS	Fatty acids	$C_{23}H_{46}O_2Si$	26.87	4.21		
1-Monopalmitin, 2TMS	Fatty acid glycerides	$C_{25}H_{54}O_4Si_2$	28.58	8.21		
Glyceryl monostearate, 2TMS	Fatty acid glycerides	$C_{27}H_{58}O_4Si_2$	31.11	4.45		
Populus x euramericana						
L-Alanine, 2TMS	Amino acids	C ₉ H ₂₃ NO ₂ Si ₂	5.62	0.43		
L-Valine, 2TMS	Amino acids	$C_{11}H_{27}NO_2Si_2$	7.71	1.27		
L-Leucine, 2TMS	Amino acids	$C_{12}H_{29}NO_2Si_2$	9.10	0.56		
L-Proline, 2TMS	Amino acids	$C_{11}H_{25}NO_2Si_2$	9.20	3.53		
Serine, 3TMS	Amino acids	$C_9H_{23}NO_3Si_2$	10.20	0.69		
L-Threonine, 3TMS	Amino acids	$C_{13}H_{33}NO_3Si_3$	10.60	0.93		
L-5-Oxoproline, 2TMS	Amino acids derivate	$C_{11}H_{23}NO_3Si_2$	12.99	5.31		
Palmitic acid, TMS	Fatty acids	$C_{19}H_{40}O_2Si$	22.74	2.04		

Linoleic acid, TMS	Fatty acids	$C_{21}H_{38}O_2Si$	24.77	3.85
Oleic acid + α-Linolenic acid	Fatty acids	$C_{21}H_{38}O_2Si$	24.84	10.26
Stearic acid, TMS	Fatty acids	$C_{21}H_{44}O_2Si$	25.12	1.22
Eicosenoic acid, TMS	Fatty acids	$C_{23}H_{48}O_2Si$	26.87	2.72
1-Monopalmitin, 2TMS	Fatty acid glycerides	$C_{25}H_{54}O_4Si_2$	28.57	12.23
Glyceryl monostearate, 2TMS	Fatty acid glycerides	$C_{27}H_{58}O_4Si_2$	31.11	6.04

Representative chromatograms are shown in Figs. 1–3, where peak patterns correspond to trimethylsilyl (TMS) derivatives, which enhance compound volatility and detection.

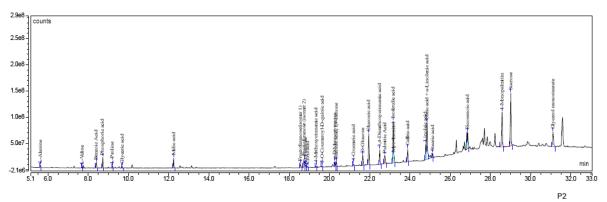


Figure 1. GC-MS chromatogram of Populus nigra L. buds after TMS derivatization.

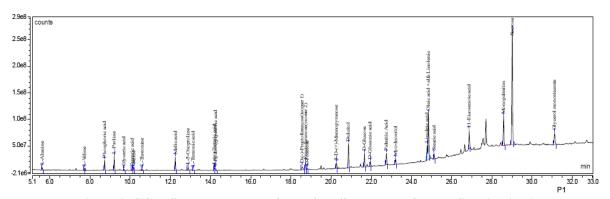


Figure 2. GC-MS chromatogram of Populus alba L. buds after TMS derivatization.

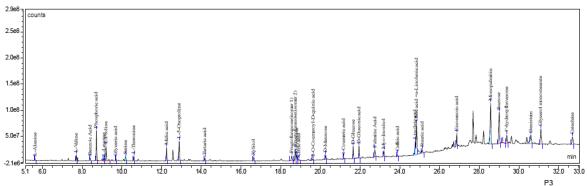


Figure 3. GC-MS Chromatogram of *Populus x euramericana* (Dode) Guinier buds after TMS derivatization.

Fig. 4 illustrates the relative content of bioactive compounds, fatty acids, and amino acids in the three poplar species.

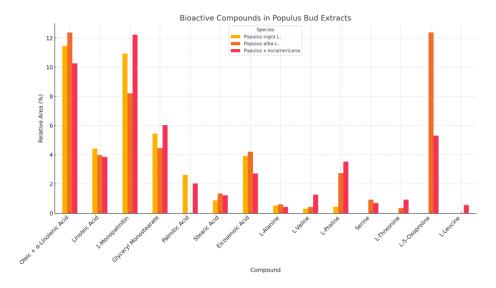


Figure 4. Comparative Analysis of Bioactive Compounds in Bud Extracts of P. nigra, P. alba, and P. \times euramericana.

3.1.2. Fatty acids assay

The quantification of fatty acids in the extracts obtained from the three poplar species (black, white, and *Euroamerican poplar*) is presented in Table 2, while Fig. 5 illustrates the fatty acid content expressed as grams per 100 grams of extract for each species.

Table 2. Fatty acid content in the buds of three Populus species.

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	Fatty acid content [g / 100 g extract]				
	Populus nigra	Populus alba	Populus x euramericana		
Palmitic acid	0.008	0.01	0.007		
Linoleic acid	0.036	0.037	0.036		
Linolenic acid	0.005	0.007	0.005		
Oleic acid	0.017	0.017	0.017		

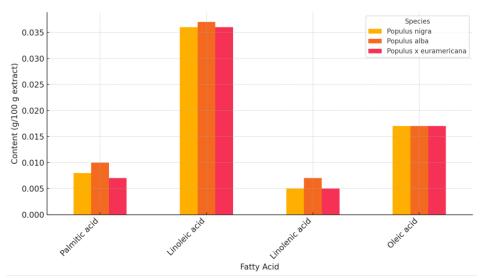


Figure 5. Fatty Acid Composition (g/100 g Extract) in Buds of P. nigra, P. alba, and P. × euramericana.

3.2. DISCUSSIONS

The GC-MS analysis of the 50% ethanolic extract from *Populus nigra* buds revealed a variety of fatty acids and amino acids, highlighting its nutritional potential and pharmacological significance.

The fatty acid profile of P. nigra was diverse and quantitatively dominant, with oleic acid and α -linolenic acid as the most abundant components (11.44%), followed by linoleic acid (4.43%), glyceryl monostearate (5.45%), and eicosenoic acid (3.92%). These unsaturated fatty acids are known for their antioxidant, anti-inflammatory, and cardioprotective properties [15,16], suggesting that P. nigra buds may have therapeutic applications. Additionally, palmitic acid (2.63%), stearic acid (0.88%), and other saturated fatty acids were also present, contributing to structural and metabolic roles in cellular function. 1-Monopalmitin, found in big amounts in black poplar's buds (10.93%), a fatty acid glyceride, is linked with its emulsifying role, to include the fatty compounds in the water content of the buds.

Among the identified compounds, amino acids such as L-alanine (0.52 %), L-valine (0.31 %), and L-proline (0.44%) were present, contributing to the development and stress defence of the poplar buds. Although they are found in small amounts, their importance in the functioning of the human body is vital, as they serve as precursors for many primary and secondary metabolites, being essential dietary components [17].

The GC-MS analysis of the 50% ethanolic extract from *P. alba* buds identified a wide range of amino acids, fatty acids, and fatty acid glycerides.

The fatty acid composition of P. alba was dominated by unsaturated fatty acids, with oleic acid and α -linolenic acid (12.38%) as the most abundant, followed by linoleic acid (3.98%) and 11-eicosenoic acid (4.21%). These polyunsaturated fatty acids (PUFAs) are known for their anti-inflammatory, cardioprotective, and neuroprotective properties, making P. alba a potential source of health-supporting lipids [18]. Additionally, palmitic acid (3.07%) and stearic acid (1.35%), two saturated fatty acids, contribute to cellular structural integrity and metabolic functions.

The identification of 1-monopalmitin (8.21%) and glyceryl monostearate (4.45%), both fatty acid glycerides, suggests the presence of lipid metabolism intermediates that may have emulsifying and moisturizing properties, supporting the potential cosmetic and pharmaceutical applications of P. alba extracts.

The amino acid profile of *P. alba* revealed notable concentrations of L-alanine (0.60%), L-valine (0.43%), L-proline (2.75%), serine (0.93%), and L-threonine (0.35%). Among these, L-proline exhibited the highest concentration (2.75%), suggesting its possible role in stress tolerance, osmo protection, and antioxidant activity within the plant [19,20]. Choudhury et al. (2024) highlighted the role of proline in reversing senescence-associated mitochondrial dysfunction and aging hallmarks [21]. The presence of serine and threonine, essential for protein synthesis and metabolic pathways [22], further highlights their importance in biochemical regulation and potential nutritional or medicinal applications. An important amino acid derivative found in an important quantity is L-5-oxoproline (12.38%), which is involved in the glutathione cycle and oxidative stress regulation. Lei et al. (2024) noticed that L-5-oxoproline can be converted to glutamate to regulate amino acid metabolism in plants, and is associated with a higher tolerance to abiotic stresses, such as salt and heat stress [23], thus, making it a promising compound that can fight climate change, and an important resource for nutraceutical compounds that can be found in the poplar's buds.

Compared to P. nigra, P. alba showed a higher concentration of L-proline and certain fatty acid glycerides, which may indicate its superior capacity for stress adaptation and lipid accumulation. The dominance of polyunsaturated fatty acids, particularly α -linolenic acid and

linoleic acid, suggests strong antioxidant and anti-inflammatory potential, reinforcing its medicinal and nutraceutical value.

Given these findings, *P. alba* buds could be explored for therapeutic applications, dietary supplements, or functional cosmetics, with further research needed to elucidate their full pharmacological benefits.

The GC-MS analysis of the 50% ethanolic extract from *P. euramericana* buds revealed a complex profile of fatty acids, fatty acid glycerides, and amino acids, indicating its nutritional and potential therapeutic value.

The fatty acid profile of P. euramericana was dominated by unsaturated fatty acids, with oleic acid $+ \alpha$ -linolenic acid $+ \alpha$ -lin

The amino acid profile of *P. euramericana* included L-alanine (0.43%), L-valine (1.27%), L-leucine (0.56%), L-proline (3.53%), serine (0.69%), and L-threonine (0.93%), alongside L-5-oxoproline (5.31%). Notably, L-proline (3.53%) and L-5-oxoproline (5.31%) were present in high amounts, suggesting an important role in environmental stress resistance, oxidative stress fighting and anti-aging protection [19-21]. These findings align with the adaptive nature of hybrid *Populus* species, which often exhibit enhanced resilience to environmental stressors compared to their parental species.

Compared to *P. nigra* and *P. alba*, *P. euramericana* demonstrated a relatively high concentration of L-5-oxoproline and glyceryl monostearate, which may contribute to its enhanced oxidative stress defence and lipid metabolism efficiency. The dominance of PUFAs, particularly α-linolenic acid and linoleic acid, suggests strong antioxidant and anti-inflammatory potential, reinforcing their therapeutic relevance in cardiovascular and neuroprotective health applications. Additionally, the presence of essential amino acids such as L-valine, L-leucine, and L-threonine highlights the nutritional value of *P. euramericana* extracts. Given these findings, *P. euramericana* exhibits a rich biochemical composition, making it a promising candidate for further pharmacological studies.

The results of the fatty acid assay indicate the presence of nutritionally important fatty acids in all three poplar species. Palmitic acid, a saturated fatty acid commonly found in plant products, was present in similar concentrations across the species, with slightly higher levels in white poplar (0.010~g/100~g extract) and the lowest in Euroamerican poplar (0.007~g/100~g extract).

Linoleic acid, an essential omega-6 polyunsaturated fatty acid, was identified in comparable amounts in all three extracts, with slight variations; the highest concentration was found in white poplar extract (0.037 g/100 g extract). This fatty acid is crucial for maintaining the cell membrane integrity and plays a significant role in biological functions such as inflammation regulation [25,26]. Linolenic acid, an important omega-3 polyunsaturated fatty acid, was detected in black poplar and Euroamerican poplar (0.005 g/100 g extract), but in much lower amounts in white poplar extract (0.0007 g/100 g extract). Omega-3 fatty acids are known for their anti-inflammatory properties and beneficial effects on cardiovascular health [27].

Oleic acid, a monounsaturated fatty acid with beneficial effects on the cardiovascular system, was detected in all three poplar species (0.017 g/100 g extract).

The presence of fatty acids and their derivatives in the buds of these species, especially the unsaturated ones, contributes to beneficial effects on neural metabolism and cell membranes, with implications for reducing inflammation, oxidative stress, and blood pressure. These effects have a positive impact on various conditions, including cardiovascular, psychiatric, skin, and musculoskeletal diseases [25-30]. The high content of unsaturated fatty acids (linolenic acid, oleic acid, and linoleic acid) in all three Romanian poplar species (over 10%) enhances their pharmacological value.

Fatty acid-rich extracts could be incorporated into fortified foods, dietary oils, or plant-based beverages to provide these health benefits. Fatty acids, especially oleic acid, linoleic acid, and glycerides, have well-documented benefits for the skin, making poplar buds promising ingredients in moisturizing and anti-aging creams, wound healing formulations, hair and scalp treatments.

GC-MS analysis confirmed that the buds of all three Populus species contain significant levels of bioactive fatty acids, glycerides, and amino acids. Among them, $P. \times euramericana$ showed the highest relative total content of fatty acid derivatives and amino acids, suggesting that it could be the most promising source for nutraceutical or cosmetic applications. The rich content of bioactive compounds recommends the exploitation of poplar buds in obtaining natural cosmetic products as a healthy alternative with beneficial effects in healing skin conditions. Effective marketing is the key to raising awareness of this [31].

4. CONCLUSIONS

This study revealed that the buds of *Populus nigra*, *Populus alba*, and *Populus* \times *euramericana* are rich in essential fatty acids and amino acids with nutraceutical potential. Key compounds such as linoleic acid, oleic acid, and α -linolenic acid were identified, highlighting their anti-inflammatory and cardiovascular benefits. Additionally, the detection of glycerides like 1-monopalmitin and glyceryl monostearate highlights their cosmetic and nutraceutical value. Among the species, *Populus* \times *euramericana* showed slightly higher concentrations of key compounds, suggesting its promise for future formulations. The findings suggest that poplar buds are a valuable natural source for future applications in health supplements, functional foods, and cosmetics.

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