



RESEARCH ARTICLE

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Purification of Ursolic Acid from the Peel of *Malus domestica* ‘Ak Saki’

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ABSTRACT

This study aimed to isolate and purify ursolic acid from the peel of *Malus domestica* ‘Ak Saki’ using a rapid, practical, and low-cost extraction–precipitation protocol, and to confirm the identity and purity of the isolated compound through chromatographic and spectroscopic analyses. Apple peels were shade-dried, and ground into powder. Ultrasonic-assisted extraction was performed using acetone in four consecutive cycles. The combined extracts were concentrated under reduced pressure, and purification was achieved via acetone-induced precipitation followed by multiple washing steps and recrystallization in hot methanol. Purity was evaluated using thin-layer chromatography (TLC), while structural confirmation was carried out through ¹H- and ¹³C-NMR spectroscopy. A total of 5.85 g of crude extract was obtained from 40 g of dried apple peel, yielding 220 mg of purified ursolic acid. The isolated compound constituted 3.76% of the crude extract and 0.55% of the dried peel material. TLC analysis showed a single well-defined spot, indicating high purity. ¹H- and ¹³C-NMR data were consistent with literature values for ursolic acid, confirming the successful isolation of a highly pure triterpenoid. *Malus domestica* ‘Ak Saki’ peel represents a rich and accessible natural source of ursolic acid. The developed extraction–precipitation procedure provides a simple, efficient, and economical method for obtaining high-purity of ursolic acid without the need for chromatographic columns. This approach may support future phytochemical research focused on ursolic acid purification.

Keywords: *Malus domestica* ‘Ak saki’cultivar, ursolic acid, isolation.

Malus domestica ‘Ak Saki’ Elma Kabuğundan Ursolik Asidin Saflaştırılması

ÖZET

Bu çalışma, *Malus domestica* ‘Ak Saki’ elma kabuğundan ursolik asidin hızlı, pratik ve düşük maliyetli bir ekstraksiyon–çöktürme protokolü ile izole edilmesini ve saflaştırılmasını; ayrıca elde edilen bileşiğin saflığının kromatografik ve spektroskopik analizlerle doğrulanmasını amaçlamıştır. Elma kabukları gölgede kurutulmuş ve toz hâline getirilmiştir. Aseton kullanılarak dört ardışık döngüde ultrasonik destekli ekstraksiyon uygulanmıştır. Birleştirilen ekstraktlar düşük basınç altında yoğunlaştırılmış; ardından aseton ile çöktürme, çoklu yıkama adımları ve sıcak metanolde rekristalizasyon yoluyla saflaştırma gerçekleştirilmiştir. Saflık ince tabaka kromatografisi (TLC) ile değerlendirilmiş, yapısal doğrulama ise ¹H- ve ¹³C-NMR spektroskopisi ile yapılmıştır. Kuru elma kabuğunun 40 gramından toplam 5.85 g ham ekstrakt elde edilmiş ve bu ekstrakttan 220 mg saf ursolik asit izole edilmiştir. İzole edilen bileşik, ham ekstraktın %3.76’sını ve kuru kabuk materyalinin %0.55’ini oluşturmuştur. TLC analizinde tek ve belirgin bir bant elde edilerek yüksek saflık doğrulanmıştır. ¹H- ve ¹³C-NMR verileri literatürde bildirilen ursolik asit değerleriyle tamamen uyumlu bulunmuş, böylece yüksek saflıkta bir triterpenoidin başarıyla izole edildiği doğrulanmıştır. *Malus domestica* ‘Ak Saki’ elma kabuğu, ursolik asit için zengin ve kolay erişilebilir bir doğal kaynak sağlamaktadır. Geliştirilen ekstraksiyon–çöktürme yöntemi, kromatografik kolon gerektirmeden yüksek saflıkta ursolik asit elde etmek için basit, verimli ve ekonomik bir yaklaşım sunmaktadır. Bu yöntem, ursolik asit saflaştırılmasına yönelik gelecekteki fitokimyasal araştırmalara katkı sağlayabilir.

Anahtar Kelimeler: *Malus domestica* ‘Ak Saki’ çeşidi, ursolik asit, izolasyon.

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1. INTRODUCTION

Apple (*Malus domestica* Borkh., Rosaceae) is among the most widely cultivated fruit species globally, with a long history of domestication and cultural diffusion. Originating from Central Asia, particularly regions home to wild species such as *Malus sieversii* and *Malus sylvestris*, the modern domesticated apple has undergone extensive genetic diversification through natural hybridization, human-mediated selection, and wide geographical dispersal (Chen et al., 2021; Spengler, 2019). Historical records indicate that apple cultivation dates back more than 4000 years, with early domestication in Mesopotamia and Egypt, followed by substantial expansion during the Roman era as orcharding practices developed and spread across Europe (Cornille et al., 2012). As agriculture evolved, apple production became integrated into both subsistence and commercial farming systems, ultimately establishing the species as a globally dominant fruit crop.

In addition to its agronomic and economic relevance, apple is highly regarded for its rich phytochemical profile. Numerous studies have shown that apples contain significant levels of phenolic acids, flavonoids, anthocyanins, vitamins, and triterpenoids, many of which are concentrated in the peel rather than the pulp (He & Liu, 2008). Consequently, apple peel has emerged as a promising natural source of bioactive compounds with nutraceutical and pharmacological potential.

Among Turkish local varieties, *Malus domestica* ‘Ak Saki’ holds particular importance. This cultivar is traditionally grown in Erzincan and its surrounding regions, where it has been cultivated for generations and carries cultural and historical significance for the local community. Unlike many commercial varieties, the phytochemical profile of traditional apple cultivars grown in Eastern Türkiye has only recently begun to be characterized. In the most comprehensive published study to date, the physicochemical properties and phenolic composition of several local apples including the ‘Ak Saki’ and ‘Karak Saki’ varieties were evaluated using HPLC-based analysis. Freeze-dried peel, pulp, and whole-fruit extracts were examined, revealing that phenolic content and antioxidant capacity vary markedly among cultivars. Phenolic profiling showed that (-)-epicatechin was particularly abundant in the peel of ‘Kara Saki’, while *p*-coumaric acid occurred in lower quantities across samples. Hydroxycinnamic acids were detected predominantly in peel tissues, suggesting that phenolic accumulation is tissue dependent. Importantly, these traditional cultivars were noted for their unusually long natural shelf life, a trait likely associated with their phenolic composition, especially ferulic acid derivatives. To date, no published study has specifically evaluated

antimicrobial, antiproliferative, or anti-inflammatory activities of ‘Ak Saki’ peel; thus, current knowledge on this cultivar is limited primarily to its phenolic profile and antioxidant potential (Gökşen & Keleş, 2020).

Ursolic acid, one of the major triterpenoids in apple peel, typically occurs at concentrations between 50–200 mg per 100 g of peel (Arulnangai et al., 2025). It is a pentacyclic triterpenoid widely distributed in plant species such as *Olea europaea*, *Rosmarinus officinalis*, *Lavandula angustifolia*, *Salvia officinalis*, and *Malus domestica* (Batiha et al., 2023; Cedillo-Portillo et al., 2024; Elhrech et al., 2024; Ghorbani & Esmailizadeh, 2017). Extensive research has described its broad spectrum of biological activities, including anti-inflammatory, antioxidant, cardioprotective, anticancer, antimicrobial, and antidiabetic/metabolic regulatory effects (Arulnangai et al., 2025; Bakry et al., 2025). Mechanistically, ursolic acid modulates NF- κ B, MAPK, PI3K/Akt, AMPK, and apoptotic signaling pathways, demonstrating strong potential as a multifunctional phytochemical agent (Arulnangai et al., 2025; Limami et al., 2023).

Despite its pharmacological significance, the practical isolation of ursolic acid from natural sources remains challenging due to matrix complexity and co-extraction of structurally similar compounds such as oleanolic acid. Many classical phytochemical purification workflows rely on solvent partitioning, chromatographic separation, or large-scale fractionation, which can be time-consuming or costly. Apple peel, however, offers cost-effective and plentiful raw material, enabling simpler extraction strategies.

The present study aims to develop a rapid, efficient, and low-cost method for the purification of ursolic acid from the peel of *Malus domestica* ‘Ak Saki’. By employing ultrasonic-assisted acetone extraction followed by a precipitation-based purification protocol, this work demonstrates that high-purity ursolic acid can be isolated without chromatographic columns. Structural and purity confirmation was achieved through TLC profiling and ^1H and ^{13}C -NMR spectroscopy. The findings highlight the potential of apple peel valorization for natural product research and provide a practical workflow suitable for both academic and industrial applications.

2. MATERIAL and METHOD

2.1. Plant Materials

Fresh fruits of the local apple cultivar *Malus domestica* Borkh. ‘Ak Saki’ (Rosaceae) were collected from a privately cultivated orchard in Elmaköy Village, Erzincan, Türkiye (1294 m altitude), during October 2024. Since the study focused on a cultivated fruit variety rather than

a wild botanical collection, a formal herbarium voucher specimen was not deposited. Botanical identification and cultivar authentication were performed by Assoc. Prof. Dr. Halil İbrahim Öztürk. Representative photographic documentation of the cultivar was recorded.

The apple peels were manually removed using a standard household fruit peeler, producing peel layers of approximately 1–2 mm thickness (Chahal & Singh, 2021). The peeled material was air-dried under shade at room temperature (25 °C) for one week and periodically aerated to prevent fungal growth and moisture accumulation. After complete drying, the peels were ground into a fine powder and stored in airtight, light-protected containers until extraction (Eweg et al., 2019).

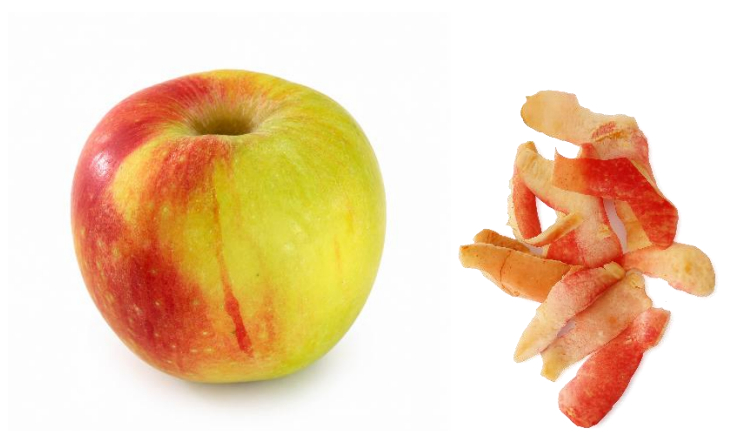


Figure 1. Representative image of the local apple cultivar *Malus domestica* Borkh. ‘Ak Saki’ (Rosaceae) and the manually separated apple peels

2.2. Preparation of Extracts

Powdered apple peel was extracted with acetone. The mixture was placed in an ultrasonic bath and sonicated for 10 min at 40 kHz to enhance extraction efficiency. The extraction was performed under non-heated ultrasonic conditions. This procedure was repeated four times. After each cycle, the extract was filtered, and fresh acetone was added to the plant residue. All filtrates were combined and concentrated under reduced pressure using a rotary evaporator at 35 °C, yielding a semi-solid crude extract (Kim & Lee, 2002).

2.3. Purification procedure

The concentrated extract was treated with excess acetone, resulting in the formation of a precipitate. The precipitate was collected and washed four times with 50 mL of acetone to remove impurities. It was then dissolved in hot methanol and left to stand overnight, allowing

the target compound to reprecipitate (Aydın et al., 2023). The resulting solid was filtered and air-dried to yield 220 mg of purified ursolic acid.

2.4. Chromatographic and Spectroscopic Analysis

Purity assessment was performed by TLC using Silica Gel 60 F₂₅₄ plates and a mobile phase of hexane: ethyl acetate (7:3, v/v). Developed plates were sprayed with ethanol: sulfuric acid (95:5) and heated at 110 °C to visualize the bands (Cường et al., 2016). Structural elucidation was conducted using ¹H- and ¹³C-NMR spectroscopy in DMSO-d₆ using Bruker Avance II 400 MHz spectrometer.

3. RESULTS and DISCUSSION

3.1. TLC Analysis and Visual Evaluation of Purity

Thin-layer chromatography (TLC) was used to compare the chemical profile of the crude acetone extract with the purified ursolic acid fraction. Figure 2 presents the TLC plates developed under identical chromatographic conditions mentioned above.

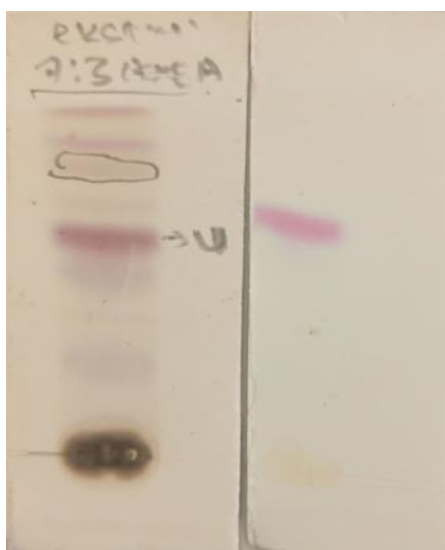


Figure 2. TLC comparison of (left) crude acetone extract of *Malus domestica* ‘Ak Saki’ peel and (right) purified ursolic acid.

The crude extract exhibited multiple bands of varying intensities distributed along the migration path. These bands reflect the presence of a complex mixture of triterpenoids, and other lipophilic components naturally occurring in apple peel. The lower portion of the plate shows a dense, darkened region indicative of strongly retained highly polar impurities, while several pink-to-red bands appearing at different retention factor values (R_f) demonstrate multiple co-extracted constituents. This heterogeneous profile confirms that the raw extract contains

numerous compounds besides the target triterpenoid. In contrast, the purified sample produced a single, well-defined pink band at the expected R_f position for ursolic acid. The absence of additional spots indicates that interfering components were successfully removed during the precipitation and washing steps. The uniformity and sharpness of the spot confirm the high purity of the isolated compound. The comparison of the two plates clearly demonstrates the effectiveness of the purification protocol: while the crude extract shows a broad chemical composition, the purified sample consists of a single spot, consistent with ursolic acid.

^1H -NMR spectrum of isolated molecule (Figure 3) displayed all characteristic resonances of ursolic acid: a distinct olefinic proton at approximately 5.14 ppm corresponding to the $\Delta^{12(13)}$ double bond, a deshielded oxygenated methine proton at C-3 in the 3.1–3.5 ppm region, and a series of sharp methyl singlets and aliphatic multiplets between 0.7–2.2 ppm representing the saturated ursane skeleton. The broad signal observed around 11.9 ppm, attributable to the carboxylic acid proton at C-28, clearly confirmed that the molecule remained in its free acid form. The absence of extraneous peaks across the aromatic and olefinic regions, along with the clean aliphatic envelope, demonstrated that the isolated compound possessed high purity. Collectively, the chromatographic and spectroscopic findings confirm that the applied extraction–precipitation protocol efficiently yielded high-purity ursolic acid from apple peel without requiring complex and time-consuming chromatographic separation.

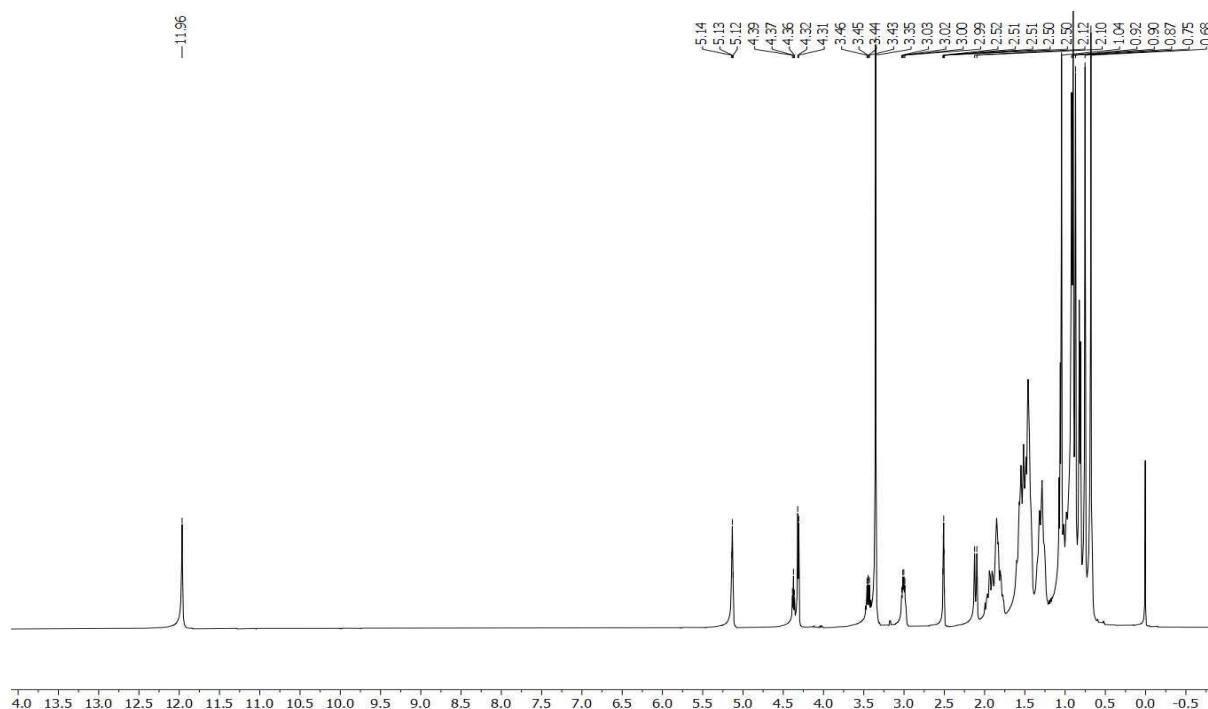


Figure 3. ^1H -NMR spectra of ursolic acid (400 MHz, in DMSO-d_6)

The ^{13}C -NMR spectrum of the purified compound (Figure 4) exhibited a total of thirty well-resolved carbon signals, fully consistent with the pentacyclic triterpenoid skeleton of ursolic acid. A prominent resonance at approximately δ 178.7 ppm corresponded to the carboxylic acid carbon (C-28), confirming the presence of the intact $-\text{COOH}$ functionality. Two additional downfield signals at around δ 138.6 ppm and δ 125.0 ppm were attributed to the olefinic carbons C-13 and C-12, characteristic of the $\Delta^{12(13)}$ double bond of the ursane framework. The oxygenated carbon at C-3, bearing the hydroxyl substituent, appeared in the expected region near δ 77.29 ppm, further supporting the identity of the triterpenoid. The remaining signals clustered between δ 15–55 ppm represented multiple methyl, methylene, and methine carbons forming the saturated, polycyclic ursane backbone. The well-defined peak distribution, absence of unexpected resonances, and perfect agreement with literature confirm the structural identity and high chemical purity of the isolated ursolic acid (Şimşek et al., 2023).

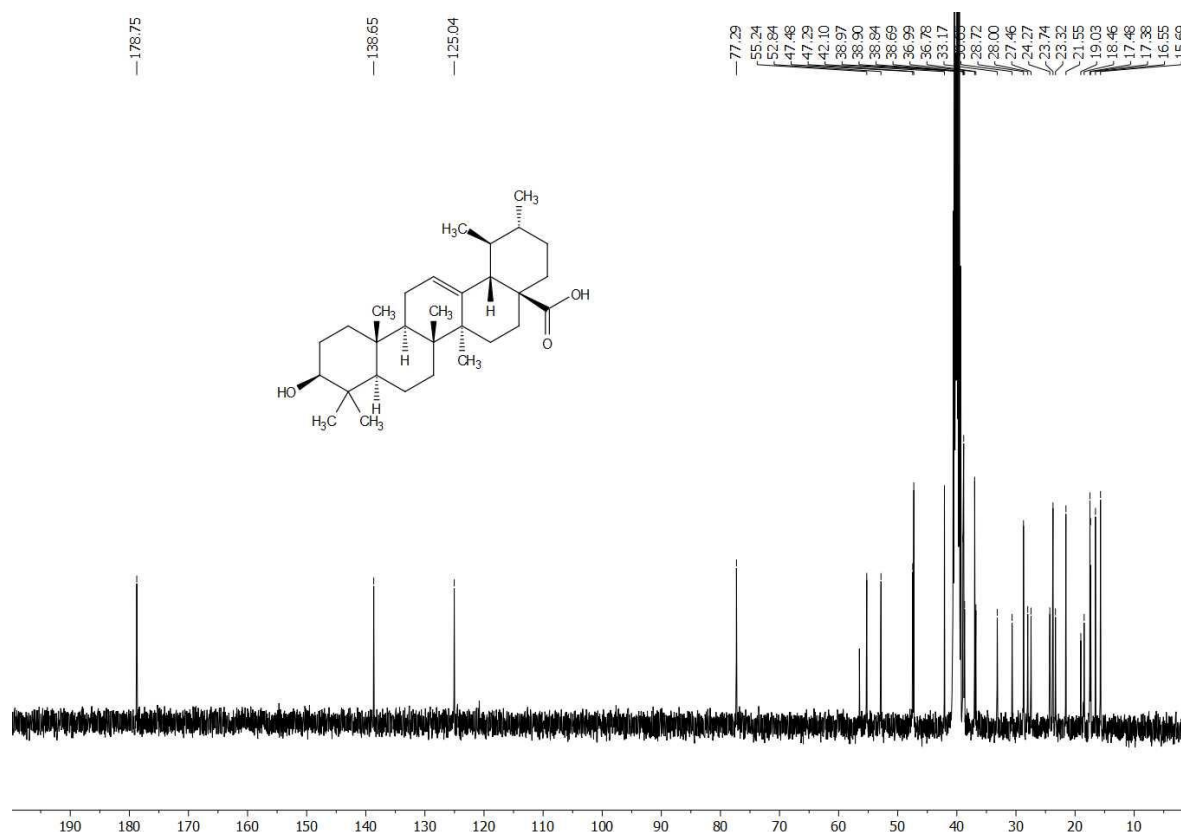


Figure 4. ^{13}C -NMR spectra of ursolic acid (100 MHz, in DMSO-d_6)

3.2. Isolation yield of ursolic acid

From 40 g of shade-dried *Malus domestica* ‘Ak Saki’ peel, the ultrasonic-assisted acetone extraction produced 5.85 g of crude extract, corresponding to an overall extraction yield of 14.6% (w/w) relative to the dry plant material. The crude extract subjected to acetone-induced precipitation and recrystallization in methanol, yielding 220 mg of purified ursolic acid. This corresponds to 3.76% ursolic acid within the crude extract and an overall 0.55% ursolic acid content in the dried apple peel. The isolation yield indicates that apple peel, often considered an agro-industrial by-product, is a relatively rich natural source of pentacyclic triterpenoids. Furthermore, the ability to recover ursolic acid through a non-column, precipitation-based protocol demonstrates the efficiency and practicality of the developed method.

The previous studies generally reports ursolic acid levels ranging from 0.05% to 0.20% in apple peels, as noted by He and Liu (2007), with broader reviews indicating similar ranges for fruit tissues (Kashyap et al., 2018; Shanmugam et al., 2013). In comparison, the present study quantified 0.55% ursolic acid in dried peel of *Malus domestica* ‘Ak Saki’, which is markedly higher than many previously published values. This difference may be associated with cultivar-specific phytochemical composition as well as regional environmental factors such as altitude, sunlight exposure, and climatic stress conditions that influence secondary metabolite accumulation. The relatively high recovery demonstrates that apple peel, commonly regarded as an agro-industrial by-product, can serve as a rich source of pentacyclic triterpenoids.

In addition to cultivar-related differences, the extraction and purification strategy may also have contributed to the elevated recovery. Ultrasonic-assisted acetone extraction likely improved solvent penetration and facilitated the release of triterpenoids from peel tissues. Furthermore, unlike conventional purification workflows that frequently require multiple chromatographic separation steps, the present protocol relied on selective precipitation and recrystallization, enabling efficient enrichment of ursolic acid with minimal processing steps and solvent consumption. The ability to isolate high-purity ursolic acid without column chromatography highlights the practical and economical nature of the developed method and suggests its potential applicability for preparative-scale triterpenoid isolation.

4. CONCLUSION

This study demonstrates that ursolic acid can be rapidly and efficiently purified from the peel of *Malus domestica* ‘Ak Saki’ using a simple extraction–precipitation protocol. The method provides high yield and purity without chromatographic instrumentation. The approach offers

a practical alternative for laboratories focusing on natural product isolation and can be adapted for larger-scale applications. Ak saki cultivar peel, an abundant agricultural by-product, thus represents an economically and environmentally advantageous source of pentacyclic triterpenoids.

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Author Contributions

Conceptualization: HA, Methodology: HA, SMK, Investigation: HA, SMK, Data curation and analysis: HA, SMK Writing – Original draft: HA, Writing – review & editing: HA, SMK

Conflict of Interest

The authors declare that they have no conflict of interest.

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