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HPLC-DAD PROFILING OF SIDR LEAVES AND ROASTED CANDLENUT FOR ALOPECIA THERAPY

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ABSTRACT

Alopecia is a dermatological disorder characterized by progressive hair loss that often affects patients' quality of life. Conventional therapies such as minoxidil and finasteride have proven effective; however, their side effects and limited tolerability have prompted the search for safer, plant-based alternatives. Ethnopharmacologically, Ziziphus spina-christi (sidr leaves) and Aleurites moluccanus (roasted candlenut seeds) have long been used in traditional hair care. Nevertheless, scientific evidence elucidating the quantitative phytochemical profile of their purified aqueous extracts and its relevance to alopecia mechanisms remains scarce. This study aimed to identify and quantify the major bioactive compounds from purified aqueous extracts of Z. spina-christi leaves and A. moluccanus seeds using High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) and to evaluate their potential implications for alopecia therapy. The powdered samples were extracted, purified by liquid–liquid partition, and analyzed using a C18 column with an acetonitrile–water gradient as the mobile phase. The results revealed that sidr leaf extract was rich in rutin hydrate (245.64 ± 0.73 mg/L), gallic acid (47.63 ± 0.43 mg/L), and quercetin (6.85 mg/L), whereas roasted candlenut seed extract contained quercetin as the predominant compound (27.00 mg/L). Rutin acts as an antioxidant and angiogenesis stimulator, gallic acid exhibits anti-inflammatory activity, and quercetin serves as a 5α-reductase inhibitor relevant to androgenic alopecia. Therefore, this study bridges a critical knowledge gap by providing quantitative evidence of the phytochemical composition of purified aqueous extracts of sidr and roasted candlenut, supporting their potential development as safe and effective phytotherapeutic candidates for alopecia management.

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INTRODUCTION

Alopecia is a hair health disorder characterised by excessive hair loss, which can affect the quality of life of those who suffer from it. Clinically, alopecia is classified into several types, including alopecia areata, androgenetic alopecia, and telogen effluvium (Messenger & Harries, 2022). Conventional therapies such as minoxidil and finasteride have proven effective; however, they often cause side effects such as scalp irritation, sexual dysfunction, or allergic reactions (Riandari et al., 2023). This has prompted the search for new natural-based therapeutic agents with improved safety profiles.

One potential source is sidr leaves (*Ziziphus spina-christi* L.) and candlenut seeds (*Aleurites moluccanus* L.). Ethnopharmacologically, sidr leaves have been traditionally used in Middle Eastern communities for skin and hair health, whereas roasted candlenuts are utilised in Southeast Asia as a traditional hair oil. Previous phytochemical studies have reported the presence of polyphenols, flavonoids, and saponins in both plants, with several investigations demonstrating antioxidant and anti-inflammatory activities relevant to hair health (Park & Lee, 2021; Sun & Shahrajabian, 2023; Usman et al., 2022). The 5% ethanolic extract of sidr leaves demonstrated significant efficacy in promoting hair growth in male albino rats (Elfasyari et al., 2025). The majority of existing studies have been confined to qualitative phytochemical screening and preliminary pharmacological activity assessments, lacking comprehensive quantitative data on the specific concentrations of key bioactive constituents.

There have been no reports of quantitative phytochemical analyses employing modern chromatographic techniques such as High-Performance Liquid Chromatography coupled with Diode-Array Detection (HPLC-DAD) on purified aqueous extracts of sidr leaves and roasted candlenut seeds. Quantitative information regarding the contents of rutin, quercetin, and gallic acid is crucial for scientifically evaluating the therapeutic potential of these plants. Moreover, the effect of the roasting process on the transformation of phenolic compounds such as the conversion of rutin into quercetin in candlenut seeds remains poorly investigated.

The novelty of this study lies in the application of HPLC-DAD to map the phytochemical profile of purified aqueous extracts, making it more relevant to traditional modes of use such as decoctions and infusions. The integration of quantitative findings with biomedical implications in alopecia provides a comprehensive scientific basis, wherein rutin is associated with angiogenesis stimulation, gallic acid with dermal papilla protection, and quercetin with 5α-reductase inhibition. Furthermore, this research establishes a new scientific foundation linking phytochemistry, biological mechanisms, and therapeutic applications in alopecia management, thereby creating opportunities for the development of plant-based phytotherapeutics supported by stronger empirical evidence (Hughes et al., 2021; Kumar et al., 2023; Onikola et al., 2025). Thus, this study not only enriches the phytochemical data of sidr and candlenut but also introduces an innovative approach to evaluating their potential as safer candidates for alopecia therapy compared with conventional treatments.

MATERIALS AND METHODS

Instruments and Materials

The instruments employed in this study included test tubes (Iwaki), micropipettes (Rainin), an analytical balance (Fujitsu FS-AR210), watch glasses, spatulas, drop pipettes, funnels (Iwaki), 10 mL volumetric flasks (Iwaki), an ultrasonic bath (Taffware Ultrasonic 40 kHz), Whatman No. 42 filter paper, and a UV–Visible spectrophotometer (Agilent Technologies Cary 8454).

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The materials used consisted of sidr (*Ziziphus spina-christi* L.) leaves and candlenut (*Aleurites moluccanus* L.) seeds obtained from the Bukittinggi region, West Sumatra, Indonesia. The chemical reagents included 70% ethanol, n-hexane, methanol (Merck), distilled water, 0.2% formic acid, acetonitrile, catechin hydrate (Sigma), gallic acid standard, rutin hydrate standard, and quercetin standard.

Procedure

The samples used in this study consisted of dried sidr (*Ziziphus spina-christi* L.) leaves and roasted candlenut (*Aleurites moluccanus* L.) seeds collected from the Bukittinggi region, West Sumatra, Indonesia. Reference standards of quercetin, rutin hydrate, and gallic acid were obtained from Sigma-Aldrich. The powdered sidr leaves were extracted using the Ultrasonic-Assisted Extraction (UAE) method with 70% ethanol as the solvent, while the roasted candlenut seed powder was extracted using the Soxhlet extraction method with n-hexane as the solvent.

The resulting crude extracts were subsequently purified using distilled water as the solvent. The purified extracts were filtered and prepared for further analysis by High-Performance Liquid Chromatography coupled with Diode-Array Detection (HPLC-DAD). Chromatographic separation was performed using a C18 stationary phase column and a gradient mobile phase consisting of acetonitrile and water containing formic acid. The flow rate was maintained at 1.0 mL/min, with detection wavelengths set at 280 nm and 360 nm. Identification and quantification of the bioactive compounds were carried out by comparing the retention times and calibration curves of the corresponding reference standards..

RESULTS AND DISCUSSION

High-Performance Liquid Chromatography coupled with Diode-Array Detection (HPLC-DAD) analysis of the purified aqueous extracts of sidr leaves and roasted candlenut seeds successfully identified three major bioactive compounds, namely rutin hydrate, gallic acid, and quercetin, as presented in Table 1 and illustrated in Figure 1.

Table 1. Concentrations of Bioactive Compounds in the Purified Extracts of Sidr Leaves and Roasted Candlenut Seeds

No.	Compound	Sidr Leaves (mg/L)	Roasted Candlenut (mg/L)
1.	Rutin hydrate	245,13- 246,16	-
2.	Gallic acid	47,32 – 47,93	-
3.	Quercetin	6,80 – 6,89	26,85 – 27, 14

The quantification results revealed that sidr leaves were rich in rutin hydrate, with a concentration of 245.64 ± 0.73 mg/L, followed by gallic acid at 47.63 ± 0.43 mg/L, and quercetin at relatively low levels ranging from 6.80 to 6.89 mg/L. In contrast, the purified aqueous extract of roasted candlenut seeds contained no detectable rutin or gallic acid but exhibited a predominant quercetin content ranging from 26.85 to 27.14 mg/L. The consistency of retention times (RT) and the high linearity of the calibration curves (R^2 > 0.999) for all three standard compounds confirm the validity and reliability of the quantification method.

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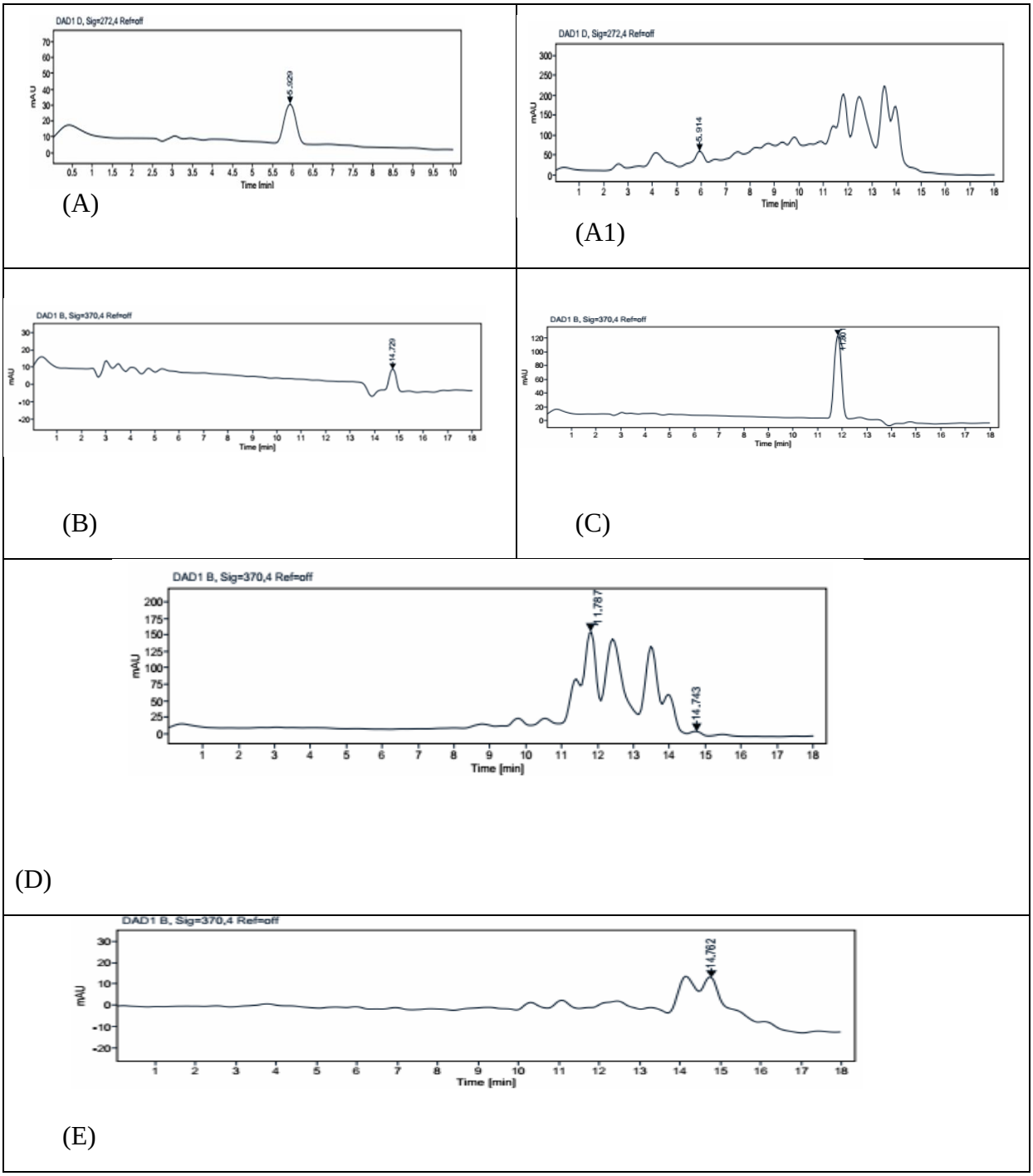

Figure 1. Chromatograms of Bioactive Compound Identification in the Purified Extracts of Sidr Leaves and Roasted Candlenut Seeds. Chromatogram of gallic acid standard (A); chromatogram of purified sidr leaf extract showing the presence of gallic acid (A1); chromatogram of quercetin standard (B); chromatogram of rutin standard (C); chromatogram of purified sidr leaf extract showing the presence of rutin and quercetin (D); chromatogram of purified roasted candlenut seed extract showing the presence of quercetin (E).

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These findings demonstrate distinct phytochemical characteristics between sidr leaves and roasted candlenut seeds. Sidr leaves were shown to be richer in glycosylated flavonoids such as rutin, whereas roasted candlenut seeds predominantly contained aglycone flavonoids such as quercetin. This profile is consistent with previous reports indicating that variations in the phytochemical composition of plants are strongly influenced by the type of plant organ, processing method, and solvent employed during extraction (Bitwell et al., 2023; Elhady et al., 2024; Susanti et al., 2024; Zaky et al., 2024). Rutin is one of the most abundant flavonoids found in the leaves of various Ziziphus species (Makhawi et al., 2020; Surendran et al., 2021), quercetin is known to be predominantly present in the seeds of plants that have undergone heat treatment (Imran & Aswad, 2025). The relatively high rutin content in sidr leaves carries important pharmacological implications. Rutin is recognised as a potent antioxidant capable of neutralising free radicals, reducing oxidative stress, and stimulating angiogenesis through the activation of the vascular endothelial growth factor (VEGF) pathway. In the context of alopecia, angiogenesis plays a crucial role in improving blood and nutrient supply to hair follicles, thereby supporting new hair growth. Moreover, in vitro studies have reported that rutin can enhance dermal papilla cell proliferation and prolong the anagen phase of the hair growth cycle (Ho et al., 2023; Oiwoh et al., 2024) (Wardhani, 2022). Therefore, the presence of rutin in sidr supports its potential use as a phytotherapeutic candidate for alopecia.

In addition to rutin, sidr also contains a considerable amount of gallic acid (approximately 47 mg/L). Gallic acid is a phenolic compound with potent anti-inflammatory, antimicrobial, and antioxidant activities. Its anti-inflammatory mechanism involves the inhibition of pro-inflammatory cytokine expression, such as TNF-α and IL-6, as well as the suppression of NF-κB pathway activation. Chronic inflammation in hair follicles is known to contribute to follicular microenvironment damage in alopecia; hence, the presence of gallic acid may help reduce hair loss associated with local inflammatory processes. Several studies have also reported that gallic acid protects keratinocyte cells from oxidative damage, further strengthening its relevance to scalp health. (Kurniawan & Zahra, 2021).

The quercetin content in sidr is relatively low (approximately 7 mg/L); however, this compound remains pharmacologically significant. Quercetin is widely recognised as an inhibitor of the enzyme 5α-reductase, which catalyses the conversion of testosterone into dihydrotestosterone (DHT), a key mediator of androgenetic alopecia. Although present in small quantities, the occurrence of quercetin in sidr may provide an additional contribution to the antiandrogenic mechanism required in the management of hair loss (Galende et al., 2024; Hughes et al., 2021; Padule et al., 2022).

In contrast to sidr, roasted candlenut seeds exhibited a predominance of quercetin at relatively high concentrations (26.85–27.14 mg/L). This level is substantially higher than that found in sidr, suggesting that the heating process during roasting may enhance the release of quercetin from its glycosidic form. Beyond its role as a 5α-reductase inhibitor, quercetin also possesses strong antioxidant activity, protecting hair follicle cells from damage induced by reactive oxygen species (ROS). Several experimental studies have demonstrated that quercetin can promote hair regrowth in testosterone-induced alopecia models in rats, showing comparable potential to finasteride in suppressing 5α-reductase activity. This finding is particularly relevant, as candlenut oil has long been traditionally used in Indonesia as a hair tonic to stimulate and nourish hair growth (Gür & Kandemir, 2022; Kurniawan & Zahra, 2021).

The validity of this study's results is reinforced by the consistent retention times matching standard references and the high coefficient of determination of the calibration

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curve, approaching 1.0 ($R^2 > 0.999$). This indicates that the HPLC-DAD method used meets the requirements for linearity, accuracy, and precision in accordance with modern phytochemical analysis guidelines. Therefore, the quantification of bioactive compounds in sidr and roasted candlenut can be scientifically justified and serves as a solid basis for further research.The implications of these findings for alopecia therapy are particularly compelling. The three identified compounds possess complementary pharmacological roles: rutin acts as an antioxidant and angiogenesis stimulator, gallic acid functions as an anti-inflammatory agent, and quercetin serves as a 5α-reductase inhibitor. Their combination has the potential to target the key mechanisms underlying androgenetic alopecia—oxidative stress, follicular inflammation, and excessive androgen activity. This concept of synergism has been widely reported in herbal therapy, where the combination of multiple bioactive compounds produces stronger biological effects than their individual use (Azizi et al., 2021; Bai et al., 2021; Kageyama et al., 2021; Muangsanguan et al., 2024; Zhao et al., 2023). These findings are also consistent with ethnopharmacological evidence. Sidr leaves have long been used in the Middle East and South Asia for hair and scalp care, while roasted candlenut is widely utilized in the Nusantara culture to promote hair growth (Ulfa, 2024).

CONCLUSIONS

The scientific data obtained through HPLC-DAD analysis in this study provide modern justification for these traditional practices. However, this research has certain limitations. The analysis was restricted to the identification and quantification of bioactive compounds using HPLC-DAD. Further studies are needed to directly evaluate the biological activities of these compounds through in vitro assays on dermal papilla cells and in vivo studies using alopecia animal models. In addition, formulation studies are required to ensure the stability and bioavailability of rutin, gallic acid, and quercetin in topical preparations such as emulgels or hair serums. Long-term safety testing is also necessary before clinical application can be recommended.

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