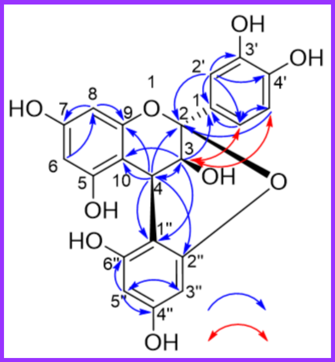

BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.bdpsjournal.org; www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088

Bangladesh J Pharmacol 2025; 20: 135-139

Identification of arachiflavanol A and proanthocyanidin A2 from the ethyl acetate extract of peanut skin

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Article Info

Received:	22 August 2025
Accepted:	22 September 2025
Available Online:	28 October 2025

DOI: 10.3329/bjp.v20i4.83866

Cite this article:
Nguyen LT, Nguyen UT, Do GH, Hoang DT, Bui LNT, Nguyen MTT, Nguyen DT. Identification of arachiflavanol A and proanthocyanidin A2 from the ethyl acetate extract of peanut skin. Bangladesh J Pharmacol. 2025; 20: 135-39.

Abstract

This study detailed the isolation and structural elucidation of two flavan-3-ol derivatives from the ethyl acetate extract of peanut skin. Structural characterization was achieved through analysis of spectral data, including HR-ESI-MS, 1D and 2D NMR, [α]D measurement, and ECD spectra, identifying the compounds as arachiflavanol A and proanthocyanidin A2. Notably, this research marks the first time arachiflavanol A has been isolated from a natural source. Furthermore, both compounds demonstrated considerable antioxidant potential in the DPPH radical scavenging assay, with IC_{50} values of 0.07 µM/mL and 0.05 µM/mL, respectively, which were comparable to the IC_{50} value of the reference antioxidant ascorbic acid (0.04 µM/mL).

Introduction

Peanut (*Arachis hypogaea* L.), a leguminous crop belonging to the family Fabaceae, is widely recognized for its edible seeds. Structurally, the peanut comprises a kernel, seed coat (commonly referred to as peanut skin), and hull. However, in most cases, only the kernels are utilized as a material for peanut-related products due to their rich source of proteins, fats, fibers, vitamins, minerals, and bioactive compounds (Hosseini Taheri et al., 2024). Meanwhile, the peanut skin is frequently regarded as an agro-waste or by-product with minimal economic value and is often discarded (Dean, 2020).

Previous studies have demonstrated that, despite accounting for only a small fraction of the total kernel mass (approximately 3%) (Dean, 2020), peanut skin harbors a substantial concentration of bioactive compounds (Bodoira et al., 2022). This unique composition has drawn significant attention from the global scientific community.

Research to date has identified various chemical constituents within peanut skin, including stilbenoids, flavonoids, phenolic acids, saponins, and triterpenes (Mingrou et al., 2022). These compounds exhibited notable biological activities (such as antioxidant, anti-inflammatory, antimicrobial, anti-cancer, and cardioprotective properties) (Mingrou et al., 2022; Cordeiro-Massironi et al., 2023). Among these, flavanols were considered a key group of bioactive compounds, characterized by the presence of A-type proanthocyanidin derivatives of catechin and epicatechin (Appeldoorn et al., 2009; Monagas et al., 2009; Dean et al., 2016). Given the diverse biological activities of these bioactive compounds, they have garnered substantial academic interest. Accordingly, this study aims to isolate and structurally characterize two flavanol derivatives from peanut skin: arachiflavanol A and proanthocyanidin A2.

Materials and Methods

Chemicals and apparatus

Compounds **1** and **2** were isolated and purified by open-column chromatography and preparative HPLC. Their structures were elucidated through 1D and 2D NMR analyses in comparison with published spectral data. Thin-layer chromatography (TLC) was performed on precoated silica gel plates (60 F254, Merck). Spots were visualized under UV light at 254 or 368 nm, and by spraying with 10% sulfuric acid, followed by heating at 105°C until clear spots appeared. Column chromatography (CC) was performed using normal-phase silica gel (230–400 mesh ASTM, Merck), reversed-phase silica gel (RP-C18) as the stationary phases. In addition, a preparative HPLC system (an Agilent 1100 series employing a YMC pack ODS-A column (250 × 20 mm, 5 µm, 12 nm)) was used to purify isolated compounds.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance NEO 600 MHz spectrometer with tetramethylsilane as the internal standard at the Institute of Chemistry, Vietnam Academy of Science and Technology. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Thermo LCQ Fleet system (Thermo Scientific, USA).

Source of material

In the present research, peanut skins were collected in November 2022 by directed peeling at manufacturing facilities for peanut-related products throughout Thai Binh Province, Vietnam.

Extract and isolation

Two kilograms of peanut (*Arachis hypogaea* L.) skin was extracted with methanol (6 L x 3 times), and the combined extract was evaporated under vacuum to yield a methanol residue. The residue was subsequently suspended in water (1 L) and then partitioned in *n*-hexane and ethyl acetate. Organic phases were collected and concentrated, yielding *n*-hexane (83.5 g) and ethyl acetate (50.0 g).

The ethyl acetate (EA) residue was further separated on a silica gel chromatography column using dichloromethane/methanol (20/1 to 1/1, *v/v*) to yield 9 fractions (EA1 to EA9). EA3 fraction was loaded on a silica gel chromatography column and eluted with solvent dichloromethane/methanol (10/1, *v/v*) to obtain 7 subfractions symbolized from EA3.1 to EA3.7. After that, subfraction EA3.5 was subjected to additional purification using silica gel CC with dichloromethane/methanol (8:1, v/v), followed by preparative HPLC employing a YMC pack ODS-A column (250 × 20 mm, 5 µm, 12 nm), a gradient solvent system (60–100% methanol in water containing 0.1% TFA) at a flow rate of 4 mL/min in 200 min yielded compound **1** (10.7 mg).

The EA8 fraction was isolated using an RP-C18 chromatography column (methanol 55% in water, *v/v*) to yield five fractions (EA8.1 to EA8.5). Then, fraction EA8.3 was further purified on a preparative HPLC system coup-ling with YMC pack ODS-A (250 × 20 mm, 5 µm, 12 nm) with gradient solvent from 10 to 40% methanol in water (0.1% TFA) with the flow rate of 4 mL/min in 180 min to obtain compound **2** (7.2 mg).

Results

The chemical structures are presented in Figure 1.

Compound **1**: white amorphous powder; [α]D25 (methanol, *c* 0.2), HR-ESI-MS: *m/z* 413.0873 [M + H]+ calc for $C_{21}H_{17}O_9$ and *m/z* 435.0654 [M+Na]+ calc for $C_{21}H_{16}O_9Na$. ^{1}H-NMR (MeOD, 600MHz): 4.120 (1H, d. *J* = 3.6 Hz, H-3), 4.268 (1H, d, *J* = 3.6 Hz, H-4), 5.985 (1H, d, *J* = 2.4 Hz, H-8), 6.009 (1H, d, *J* = 2.4 Hz, H-6), 6.035 (1H, d, *J* = 2.4 Hz, H-5''), 6.103 (1H, d, *J* = 1.8 Hz, H-3''), 6.847 (1H, d, *J* = 8.4 Hz, H-2'), 7.063 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 7.175 (1H, d, *J* = 1.8 Hz, H-5'). ^{13}C-NMR (MeOD, 150MHz): 29.35 (C-4), 67.64 (C-3), 96.34 (C-8),

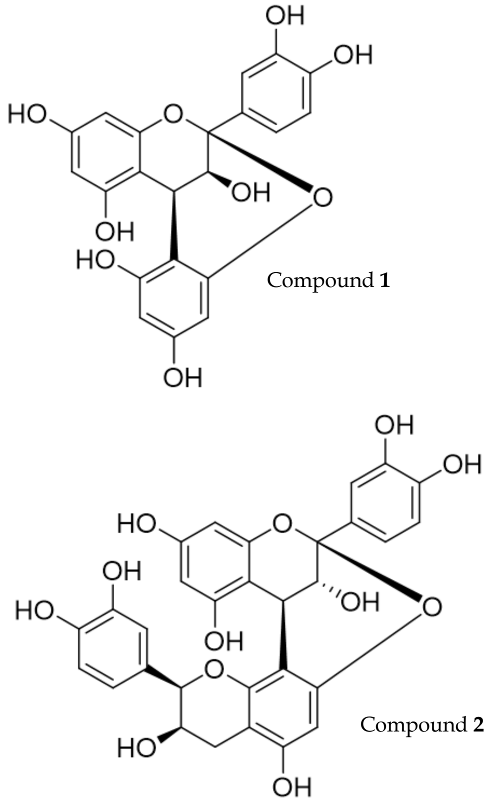

Figure 1: Structures of compound **1** and compound **2**

96.34 (C-3''), 97.6 (C-6), 97.6 (C-5''), 100.58 (C-2), 104.52 (C-10), 107.58 (C-1''), 115.65 (C-2'), 115.79 (C-5'), 119.93 (C-6'), 132.27 (C-1'), 145.67 (C-3'), 146.83 (C-4'), 154.14 (C -9), 154.14 (C-2''), 155.86 (C-4''), 158.06 (C-5), 158.06 (C-6''), 158.11 (C-7).

Further 2D NMR correlations (HSQC, HMBC, and NOESY) supported the proposed structure of compound 1. The key HMBC and NOESY interactions are shown in Figure 2, confirming that compound **1** is a flavan-3-ol derivative. The relative configuration was assigned from NOESY correlations, where the presence of cross-peaks between H-3/H-5 and H-6', together with the absence of correlation between H-3/H-3'', indicated a 3,4-cis configuration. The absolute configaration was determined from the ECD spectrum, which exhibited positive Cotton effects at 218 nm ($\Delta\varepsilon$ = +5.48) and 240 nm ($\Delta\varepsilon$ = +2.03). On this basis, compound 1 was identified as a flavan-3-ol derivative with an aromatic substituent at C-4 and a 2→O→2'' interflavan linkage.

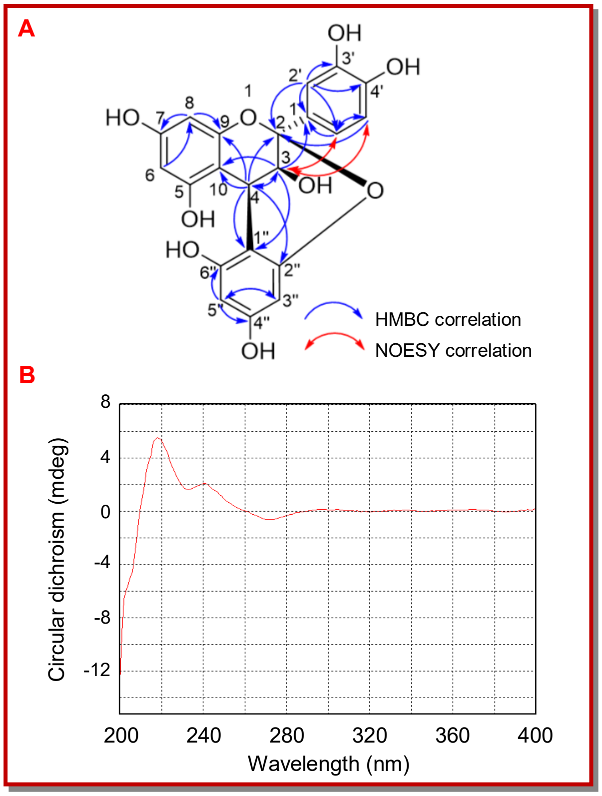

Figure 2: Several HMBC and NOESY correlations of compound **1** (A); ECD spectrum of compound **1** (B)

The antioxidant properties of two isolated flavanols were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, based on a modified version of an established protocol (Yıldırım et al., 2003). Arachiflavanol A and proanthocyanidin A2 demonstrated potent antioxidant activity, with IC$_{50}$ values of 0.07 μM/mL and 0.05 μM/mL, respectively, which were comparable to the IC$_{50}$ value of the reference antioxidant ascorbic acid (0.04 μM/mL).

Discussion

In this study, two flavan-3-ol derivatives, arachiflavanol A and proanthocyanidin A2, were successfully isolated and structurally characterized. Notably, arachiflavanol A was identified for the first time in nature. Both compounds exhibited free radical scavenging antioxidant activity with IC$_{50}$ values comparable to that of the positive control (ascorbic acid)

In contrast to previous studies, which have primarily reported the presence of common flavan-3-ols and proanthocyanidins from peanut skin, the present work describes the discovery of arachiflavanol A in nature. Earlier investigations have also focused on A-type and B-type proanthocyanidins or catechin/epicatechin derivatives isolated from *A. hypogaea* or related species, without identifying this unique flavan-3-ol structure. In the present study, arachiflavanol A was successfully isolated from the ethyl acetate extract of peanut skin using column chromatography and prep-HPLC. Its structure was comprehensively elucidated by integrating MS, 1D, and 2D NMR, and ECD analyses, and further confirmed by comparison with previously published spectral data (Sharma et al., 2015).

The HR-ESI-MS of compound **1** showed ion peaks at *m/z* 413.0873 [M + H+] (calc for $C_{21}H_{17}O_9$), and a sodium adductive ion [M+Na]+ at *m/z* 435.0654 (calc for $C_{21}H_{16}O_9Na$), indicating a molecular formula of $C_{21}H_{16}O_9$ for compound **1** with an IHP (index of hydrogen deficiency) of 14. The ^{1}H-NMR of compound **1** showed signals corresponding to seven olefinic protons (δH 5.985 (1H, d, J = 2.4 Hz, H-8), 6.009 (1H, d, J = 2.4 Hz, H-6), 6.847 (1H, d, J = 8.4 Hz, H-2'), 7.063 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 7.175 (1H, d, J = 1.8 Hz, H-5'), 6.035 (1H, d, J = 2.4 Hz, H-5''), 6.103 (1H, d, J = 1.8 Hz, H-3'')) one oxygenated methine proton (δH 4.120 (1H, d. J = 3.6 Hz, H-3)), one methine group (δH 4.268 (1H, d, J = 3.6 Hz, H-4)). The ^{13}C-NMR and HSQC spectra of compound **1** revealed 21 carbons, including 18 aromatic carbons (δC from 96.34 to 158.11), one oxygenated quaternary carbon (δC 100.58), one oxygenated methine (δC 67.64), and one methine (δC 29.35). Compared with the previous spectral data of catechin (Imtiaz et al., 2023) and epicatechin (Choi et al., 2018), the signals of aliphatic hydrogen [4.120 (1H, d, J = 3.6 Hz, H-3), 4.268 (1H, d, J = 3.6Hz, H-4)] and ^{13}C resonances [100.58 (C-2), 67.64 (C-3), 29.35 (C-4)] also appeared on NMR spectra of compound **1**, revealed the presence of flavan-3-ol structure in this compound. The positions of hydroxyl-substituted groups in aromatic systems were determined based on chemical shifts of aromatic carbons. Particularly, chemical shifts of C-5, C-7, C-3', and C-4' shifted to the upper field (from 145 ppm to 158 ppm) in comparison with aromatic carbon atoms at other positions in the molecule (from 96 ppm to 120 ppm).

However, the differences between the NMR signals of compound **1** and the aforementioned flavan-3-ol compounds (catechin and epicatechin) lay in the presence including two *meta* protons with signals on the ^{1}H-NMR spectrum at δH 6.035 (1H, d, *J* = 2.4 Hz, H-5''), 6.103 (1H, d, *J* = 1.8 Hz, H-3'') and on the ^{13}C-NMR spectrum at δC 96.34 (C-3''), 97.6 (C-5''), 107.58 (C-1''), 154.14 (C-2''), 155.86 (C-4''), 158.06 (C-6''). The existence of bond 4→1'' was proven through HMBC correlation from H-4 to C-1'', C-2'', and from H-3 to C-1'' và C-2''. The signals of C-2, C-3, and C-4 shifted to the upper field compared to the corresponding C signal in catechin or epicatechin, but in a similar range of carbon atoms in compound **2** (proanthocyanidin A2) (Nam et al., 2015). Moreover, chemical shifts of protons at δH 6.035 (1H, d, *J* = 2.4 Hz, H-5''), 6.103 (1H, d, *J* = 1.8 Hz, H-3'') and signal of aromatic carbons C-1'' to C-6'' together with calculated molecular formula $C_{21}H_{16}O_9$ and IHP value confirmed the presence of link 2→O→2'' in the planar structure of compound **1**. Compared with the reported spectral data (Sharma et al., 2015), the planar structure of compound **1** was elucidated as a derivative of flavan-3-ol with an aromatic substituted group at the C-4 position and a bridge of 2→O→2''.

The combined use of NOESY correlations and Cotton effect values provided definitive evidence for its relative and absolute configuration. While NOE correlations allow the conformational relative configuration, the absolute configuration of this flavan-3-ol was determined based on [*a*]D value and effects on the ECD spectra, combined with reported data. The presence of NOE correlation H-3/H-5 and H-6', but lack of interaction H-3/H-3'', was considered to be a diagnosis of 3,4-*cis* configuration (Bilia et al., 1996). Additionally, the positive Cotton effects of compound **1** were collected at wavelengths of 218 nm (D*e* = +5.48) and 240 nm (D*e* = +2.03), similar to those of several oligomeric A-type proanthocyanidins (Ting et al., 2014; Nam et al., 2015; Nam et al., 2017), but in contrast with the CE of the (2*β*, 4*β*)-configuration in the structure of ent-epiafzelechin (2*a* → *O* → 7, 4*a* → 8) afzelechin (Bilia et al., 1996). As a result, the absolute configuration at positions C-2, C-3, and C-4 should be 2*R*, 3*S*, 4*R*, and compound **1** was named arachiflavanol A.

The structure of compound **2** was confirmed as proanthocyanidin A2 by comparing 1D and 2D-NMR spectral data with previously reported studies (Nam et al., 2015).

These findings therefore expand the chemical profile of peanut skin, highlighting the occurrence of a structurally distinct flavan-3-ol derivative that has not been reported in earlier literature. Using advanced nuclear magnetic resonance (NMR) spectroscopy methods, along with [α]D, ECD analyses, and comparisons with previously reported data, the chemical structures and absolute configurations of arachiflavanol A and proanthocyanidin A2 isolated from peanut skin were determined. The results revealed that both isolated compounds were flavan-3-ol derivatives, a major group of bioactive compounds found in peanut skin (Appeldoorn et al., 2009; Monagas et al., 2009; Dean et al., 2016). The planar structure of arachiflavanol A has been previously described (Sharma et al., 2015). This marks the discovery of arachiflavanol A in nature and the inaugural determination of its absolute configuration through advanced spectroscopic techniques.

The detection of arachiflavanol A for the first time in nature may be attributed to several factors. First, variations in plant genotype and growth conditions could influence the biosynthetic pathways of flavan-3-ols, leading to the accumulation of this rare derivative in peanut skin. Second, the choice of extraction solvent (ethyl acetate) and the use of successive chromatographic techniques may have enhanced the recovery of minor constituents that were overlooked in earlier studies. Third, advances in spectroscopic methods, particularly high-resolution MS, 2D NMR, and ECD, provided higher sensitivity and accuracy in structural elucidation compared with the techniques available in previous decades. Collectively, these factors may explain why arachiflavanol A was not reported in earlier research and underscore the importance of methodological and technological improvements in natural product discovery.

Previous studies have also indicated that procyanidins, specifically A-type proanthocyanidins, are among the main compounds found in peanut skin extract (Bodoira et al., 2022), possessing important bioactivities such as antioxidant, anti-aging, and anti-inflammatory effects (Bodoira et al., 2022; Li et al., 2025). In this study, both isolated compounds were identified as potential antioxidants, with IC_{50} values comparable to that of the positive control, ascorbic acid. This consequence was similar to the findings reported in the previous study on the bioactivities of proanthocyanidin A2 (Lee et al., 2010). These findings contribute to the broader understanding of the chemical composition of peanut skin and highlight its potential as a source of bioactive compounds.

This study was limited to the chemical investigation of flavan-3-ol derivatives from the ethyl acetate extract of peanut skin. The biological activities of both the crude extract and the isolated compounds were assessed only through *in vitro* antioxidant assays. Although these findings provide useful preliminary data, they do not fully reflect the biological relevance and complexity of *in vivo* systems.

Another noteworthy limitation relates to the novelty of arachiflavanol A, which was identified in nature in this study. While this represents a significant advancement, the biosynthetic origin and metabolic regulation of this unique compound remain unknown. Elucidating the biosynthetic pathway of arachiflavanol A will not only deepen our understanding of plant secondary metabolism in peanut skin but also create opportunities for metabolic engineering, sustainable production, and potential applications of this compound in functional foods or therapeutic development.

Conclusion

Two flavan-3-ol derivatives, arachiflavanol A and proanthocyanidin A2, were isolated and structurally characterized from peanut skin, with arachiflavanol A reported for the first time from nature. Moreover, both showed strong antioxidant activity comparable to that of ascorbic acid, highlighting their potential as valuable bioactive constituents.

Financial Support

This work was funded by the Vietnam Academy of Science and Technology under the grant number THTETN.09/24-25.

Conflict of Interest

Authors declare no conflict of interest

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