Process Biochemistry 168 (2026) 201–213

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Upcycling peanut skin through the valorisation of its biological properties

Tiago Barros Afonso [a,*], Teresa Bonifácio-Lopes [a], Tânia Ribeiro [a], Eduardo M. Costa [a], Ana Filipa Costa [b], Daniel Mendanha [b], Tiago Macedo [c], Joana Moreira [c], Juliana A.S.A. Oliveira [c], Carla J. Silva [b], Manuela Pintado [a]

[a] CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto 4169-005, Portugal
[b] Centro Tecnológico das Indústrias Têxtil e do Vestuário de Portugal – CITEVE, Rua Fernando Mesquita 2785, 4760-034 Vila Nova de Famalicão, Portugal
[c] Centro de Nanotecnologia e Materiais Técnicos, Funcionais e Inteligentes – CeNTI, Rua Fernando Mesquita 2785, 4760-034 Vila Nova de Famalicão, Portugal

ARTICLE INFO

Keywords:
Peanut skin
Antioxidant activity
Antimicrobial activity
Phenolic compounds
Procyanidins
Skin-related applications

ABSTRACT

The industrial processing of peanuts generates large amounts of underutilized by-products, mainly skins and shells. Peanut skins, rich in bioactive compounds with antioxidant and antimicrobial properties, are promising for skin-related applications. This study investigates how different extraction technologies—ultrasound-assisted extraction (UAE), hydroethanolic, alkaline, and aqueous extraction—affect extract composition, antioxidant and antimicrobial capacities, and their impact on skin cell metabolism and antioxidant activity. UAE yielded the highest phenolic content (480.93–512.93 mg GAE/g DE), followed by hydroethanolic and alkaline methods. A similar trend was observed in the ORAC assay (2770.17–2925.00 μmol TE/g DE). LC-ESI-QqTOF-HRMS tentatively identified type-A and type-B procyanidins as major compounds. Alkaline extracts exhibited the strongest antimicrobial activity against S. aureus (MIC: 5 mg/mL) and S. epidermidis (2.5 mg/mL), while hydroethanolic and UAE extracts were active at 20 mg/mL; aqueous extracts were inactive. No significant antifungal effects were observed against filamentous fungi, but alkaline and UAE extracts inhibited *Malassezia furfur*, a skin-associated yeast linked to common dermatological conditions (MICs <1.25 and 2.5 mg/mL). In HaCaT cells, hydroethanolic extracts showed the lowest cytotoxicity (500 μg/mL), while UAE displayed the highest (62.5 μg/mL). All extracts reduced iROS formation. These results highlight peanut skin's potential in skin-related products and textiles, warranting further process optimization for scale-up.

1. Introduction

The agro-food industry produces high amounts of by-products that, despite their nutritional and bioactive potential, often remain underutilized or are relegated to low-value applications such as animal feed or biomass combustion [1]. In recent years, increasing efforts have been made toward the valorisation of these by-products to develop sustainable, cost-effective, and environmentally friendly alternatives for different industrial sectors. Some of these by-products are an excellent source of plant-derived phenolic compounds, which are well known for their antioxidant, antimicrobial, and other functional properties. Consequently, these compounds have attracted growing interest for applications in food preservation, pharmaceuticals, cosmetics, packaging, and textile industries [2–4]. Sourcing these compounds from food by-products represents a strategic approach to reduce waste and provide natural, abundant, and economically viable sources of bioactive ingredients, thus contributing to sustainable resource utilization and circular economy practices [1].

One agro-industrial by-product with high valorisation potential is peanut (*Arachis hypogaea* L.) skin, a residue obtained during the processing of peanuts of various food products, such as blanched peanuts, roasted peanuts, peanut butter, among other confectionery goods [5,6]. Although traditionally considered waste, peanut skin is now recognized as one of the most valuable peanut by-products due to its high phenolic content and bioactive potential. Consequently, it represents a promising feedstock for the recovery of phenolic compounds, including flavonoids,

* Corresponding author.
E-mail addresses: tbafonso@ucp.pt (T.B. Afonso), mvlopes@ucp.pt (T. Bonifácio-Lopes), tribeiro@ucp.pt (T. Ribeiro), emcosta@ucp.pt (E.M. Costa), afcosta@citeve.pt (A.F. Costa), dmendanha@citeve.pt (D. Mendanha), tmacedo@centi.pt (T. Macedo), jmoreira@centi.pt (J. Moreira), jaolveira@centi.pt (J.A.S.A. Oliveira), cjsilva@citeve.pt (C.J. Silva), mpintado@ucp.pt (M. Pintado).

https://doi.org/10.1016/j.procbio.2026.06.017
Received 3 February 2026; Received in revised form 27 June 2026; Accepted 28 June 2026
Available online 29 June 2026

and the development of sustainable bio-based products within integrated biorefinery and circular bioeconomy strategies [6–9]. In addition, peanut skin extracts have shown antimicrobial potential against a variety of bacteria and fungi, which suggests a potential role in replacing synthetic preservatives [8,10,11]. Previous studies have also shown that peanut skin extracts can inhibit LDL-cholesterol oxidation, protect against DNA strand breakage, and exert anti-inflammatory effects [12]. The presence of anthocyanins, flavanols, and stilbenes in these extracts further enhances their potential as functional ingredients for a range of health-related applications [5,10].

Beyond food-related uses, peanut skin-derived phenolic compounds have attracted interest for potential applications in cosmetic and textile-related fields due to their antioxidant and antimicrobial properties [13]. In skin-related applications, these properties may contribute to protection against oxidative stress and microbial imbalance. In parallel, the growing demand for sustainable and bio-based textile finishing has stimulated research into the use of natural plant extracts to impart multifunctional properties such as UV protection, antimicrobial resistance, and antioxidant capacity [14–16]. Peanut skin-derived phenolic compounds not only provide natural coloration but also serve as reducing and stabilizing agents for *in situ* synthesis of nanoparticles, enabling the development of high-performance textiles with enhanced durability and bioactivity [14]. The use of green and sustainable solvents in extraction processes aligns with the principles of green chemistry, reducing reliance on synthetic products and hazardous chemicals while supporting the development of environmentally friendly functional materials.

In this context, the present study aims to provide a comprehensive comparison of different extraction methodologies applied to peanut skin, including ultrasound-assisted extraction, to evaluate how extraction conditions influence phenolic composition and associated biological activities. Among the extraction methods evaluated, alkaline extraction was included due to its potential to promote transformation of phenolic compounds in plant matrices [17]. Although several extraction approaches have been investigated for peanut skin valorisation, reports evaluating alkaline extraction of peanut skin are scarce. Therefore, its inclusion provides insight into how extraction-induced compositional changes affect the functionality of peanut skin extracts. Differences in extraction conditions may lead to distinct phenolic profiles and consequently influence the biological properties of the resulting extracts. Although ultrasound-assisted extraction has previously been applied to peanut skin, existing studies have largely focused on the targeted recovery of specific compound classes, such as procyanidins or stilbenoids, under narrowly defined extraction conditions [18,19]. In contrast, the present work does not pursue the selective isolation of a single compound or compound family but adopts a broader, comparative approach. By integrating extraction yield analysis, detailed LC–MS phenolic profiling, antioxidant capacity, antimicrobial and antibiofilm activity, and cellular assays, this study seeks to relate compositional differences among extracts to their biological performance. This integrative strategy provides new functional insights into how extraction methodology affects phenolic composition and biological performance, supporting peanut skin valorisation as a sustainable source of bioactive compounds for skin-related applications and other bio-based industrial uses. In doing so, it contributes to a more holistic understanding of how extraction strategies shape bioactivity beyond compound-specific recovery.

2. Materials and methods

2.1. Materials

Peanut skin was obtained as a by-product from the food supplement sector, kindly supplied by Prozis (Esposende, Portugal). After receipt, the residue was stored protected from direct light, at room temperature (≈ 25 °C) and at a relative humidity of 55–65%.

2.2. Extraction methodologies

2.2.1. Conventional solvent extractions

For the conventional solvent extractions, peanut skin was extracted with deionized water (H_2O) or hydroethanolic solution (EtOH) – ethanol–water (70:30 v/v). The aqueous extraction was performed with a residue-to-solution of 10% (m/v) for 15 min at 50 °C with agitation in a thermomixer (Vorwerk, Germany). The hydroethanolic extraction was performed with a residue-to-solution of 3% (m/v) with three consecutive extractions at room temperature with agitation at 450 rpm (KS 130 Basic, IKA-Werke GmbH & Co. KG, Germany). The first extraction was done for 30 min, followed by two 15-minute extractions. After each extraction step, the samples were centrifuged (Multifuge X1, Thermo Fisher Scientific, Waltham, MA, USA) and the liquid phase recovered. At the end of both extractions, the solutions were filtered, and the ethanol was evaporated using a rotary evaporator (Buchi R−210, Buchi Labortechnik AG, Flawil, Switzerland). Samples were then lyophilized (30–50 mL per individual plastic vials) using a Christ Gamma 1–16 LSCplus freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The process was performed with a condenser temperature of –63 °C, a vacuum pressure of 0.10 mbar, and a total duration of 72 h. The shelf temperature gradually increased and reached a maximum of 25 °C during the process.

2.2.2. Ultrasound-assisted extraction

Peanut skin ultrasound-assisted extraction (UAE) was performed using a hydroethanolic mixture (50:50 v/v) as extraction solvent. The extraction was carried out on both unprocessed (as received) (UAE#1) and milled peanut skin (≤ 0.5 mm, using a Fritsch Pulverisette 19 cutting mill (Idar-Oberstein, Germany)) (UAE#2). To achieve a 3% (m/v) extraction ratio, 4.5 g of peanut skin were combined with 150 mL of solvent. The mixture then underwent ultrasound treatment, under magnetic stirring, using a Hielscher Ultrasonics GmbH UIP1000hdT device (Teltow, Germany), equipped with a titanium Hielscher sonotrode BS4d22 (frontal area: 3.8 cm^2). The system operated at 20 KHz and was programmed to work at an amplitude of 60 μm and ensure an energy density of 6.6 kJ/L. Subsequently, the mixture was stirred for 30 min on a heating plate at 50 °C. Afterward, it was centrifuged at 9000 rpm for 10 min, using a Thermo Scientific™ Sorvall™ LYNX 6000 centrifuge (Waltham, MA, USA), and filtered through a 5 μm - 13 μm filter. Afterwards, the ethanol was evaporated (Buchi R−210), and the remaining extract was lyophilized to obtain a dried extract.

2.2.3. Alkaline-assisted extraction

Peanut skin was extracted using a 0.3 M sodium hydroxide (NaOH) solution containing 20% (v/v) ethanol. The solution was prepared by dissolving granulated NaOH in water and adding absolute ethanol in a 1:4 (v/v) ratio. The extraction was performed with a residue-to-solution of 10% (m/v) for 60 min at 50 °C, with agitation at 25 rpm, using a Mathis LABOMAT (Mathis AG, Germany). Subsequently, the ethanol was evaporated (Buchi R−210), and the remaining extract was lyophilized to obtain a dried extract.

2.2.4. Extraction yield

The extraction yield was calculated based on the initial amount of peanut skin (g) used in the extraction (Equation 1) and the amount of dried extract obtained.

$$\text{Yield (\%)} = \text{weight of dried extract (g)} / \text{initial weight of peanut skin (g)} \times 100$$

2.3. Total protein quantification

The protein content was determined through the micro-Kjeldahl

method, using ISO 1871:2009 [20]. Briefly, about 0.2 g of sample was digested in a mineralization block (VELP Scientifica SRL, Usmate Velate, Italy), with concentrated sulfuric acid (96% w/w) in the presence of a catalyst. From the quantity of ammonia produced, the nitrogen content (N) was calculated and converted to protein using the conversion factor 5.46 [21]. A blank determination was also performed. Each sample was evaluated in triplicate.

2.4. Total carbohydrates (sugar) quantification

For the total carbohydrate content, the Phenol-Sulfuric Acid colorimetric method was performed following the method described by [22] adapted to a 96-well microtiter plate. Briefly, 20 mg of each extract was suspended in 2 mL of distilled water (10 mg/mL). Then, 50 μL of each sample (or its respective dilution if necessary) was mixed rapidly in a well of a 96-well microtiter plate with 150 μL of concentrated sulfuric acid (95–97%) (Honeywell, Charlotte, NC, USA). Next, 30 μL of a phenol working solution (5%) was added to the mixture and heated for 5 min at 90 °C. After cooling to room temperature for another 5 min in a water bath, the microplate was wiped dry, and the absorbance was measured at 490 nm (Synergy H1, Biotek, Winooski, VT, USA) using distilled water as a blank. Glucose was used as a standard, and the results were expressed as mg GE/g DE. Each sample was evaluated in triplicate.

2.5. Quantification of total phenolic content

For the determination of the total phenolic content (TPC), 20 mg of each extract was suspended in 2 mL of distilled water (10 mg/mL). Three replicates were performed. The TPC was determined using the Folin-Ciocalteu method, as described by Vilas-Boas et al. [23]. Briefly, the Folin–Ciocalteu reagent (100 μL, 20% (v/v) was added to the extract suspension (30 μL) and then to sodium carbonate (100 μL, 7.4% w/v) and allowed to react in the dark at room temperature for 30 min. The absorbance was then measured at 765 nm (Synergy H1) in a 96-well microplate (Sarstedt, Numbrecht, Germany). Gallic acid was used as a standard, and the results were expressed as mg GAE/g DE.

2.6. Quantification of total flavonoid content

For the determination of the total flavonoid content (TFC), 20 mg of each extract were suspended in 2 mL of distilled water (10 mg/mL). All analyses were performed in triplicate. The TFC was determined via the aluminium chloride colorimetric method following Herald et al. [24]. Briefly, 62.5 μL of sample, catechin, or solvent was added to an Eppendorf containing 275 μL of NaNO2 (0.066 M), vortexed, and incubated for 5 min. Then, 37.5 μL of $AlCl_3$ (0.75 M) was added to the mixture and vortexed. After 6 min of incubation, 250 μL of NaOH (0.5 M) was added and vortexed. Finally, 250 μL of each mixture was transferred in duplicate to a 96-well microplate, and the absorbance was measured at 510 nm (Synergy H1) using distilled water as a blank. Catechin was used as a standard for the calibration curve, and the results were expressed as mg CAE/g DE.

2.7. Identification of phenolic compounds

The phenolic compounds present in the peanut skin extracts were characterized by liquid chromatography coupled to electrospray ionization quadrupole time-of-flight high-resolution mass spectrometry (LC-ESI-QqTOF-HRMS) using a Bruker Daltonics system (Billerica, MA, USA) following the methodology described by Vilas-Boas et al. [23] with some modifications. The extract was prepared by dissolving it in ultrapure water (10 mg/mL) and adding 1 mL of cold MeOH (−80 °C) to precipitate the proteins. It was then diluted twenty times and filtered through a 0.22 μm nitrocellulose membrane before analysis. The separation was achieved in a Bruker Elute series liquid chromatograph, coupled to an UHR-QqTOF mass spectrometer with 50,000 full-sensitivity resolution (FSR) (Impact II, Bruker Daltonics, Bremen, Germany), using a BRHSC18022100 intensity Solo 2 C18 column (100 × 2.1 mm, 2.2 μm, Bruker). High-resolution mass spectrometry was used to identify the compounds. The elemental composition for the compound was confirmed according to accurate mass and isotope distribution analysis (mSigma, Bruker Daltonics). The precise mass measurement was within 5 mDa of the assigned elemental composition, and mSigma values under 20 provided confirmation. Compounds were tentatively identified by assessing the accurate mass [M-H]−.

2.8. Determination of antioxidant activity

The antioxidant activity was assessed by the oxygen radical absorbance capacity (ORAC). The ORAC assay was selected because it measures the scavenging capacity against peroxyl radicals, which are highly relevant in biological systems, while accounting for both reaction kinetics and stoichiometry. Consequently, it provides a biologically relevant evaluation of antioxidant activity. Lyophilized extracts were dissolved in distilled water (10 mg/mL) prior to analysis. The assay was done as described in the literature with some modifications [25]. Fluorescein and 2,2′-azobis(2-amidi-nopropane) dihydrochloride (AAPH) were used as fluorescent probe and peroxyl radical generator, respectively, and the reaction was conducted at 37 °C using a black polystyrene 96-well microplate (Sarstedt, Numbrecht, Germany). Each well contained 200 mL of phosphate buffer (75 mM and pH 7.4), 20 μL of blank, sample, or Trolox (1 μM–8 μM), and 120 μL of fluorescein (70 nM). Prior to the addition of AAPH (60 μL; 12 mM), the mixture was preincubated for 10 min. Fluorescence measurements were recorded at 1-minute intervals for a total duration of 90 min using an excitation wavelength of 485 nm with emission at 528 nm (Synergy H1). The microplate was automatically shaken before each reading. This assay was performed in triplicate, and the results are expressed as micromole Trolox equivalents per gram of dry extract (μmol TE/g DE).

2.9. Determination of antibacterial and antifungal activity

2.9.1. Antibacterial activity

The antibacterial activity of the extracts was assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution method as previously described [26]. Antibacterial activity was assessed against two Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 27853) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* DSM 20044). Crude lyophilized extracts were tested without further purification or fractionation. Briefly, extracts were dissolved in Mueller Hinton (MH) broth (40 mg/mL) and filtered through a 0.22 μm filter (Clarify, Phenomenex, Torrence, CA, USA). For the alkaline extract, the pH of the reconstituted stock solution was adjusted to pH 7.4 prior to antimicrobial testing. To maintain the intended final concentration, the extract was initially prepared at a slightly higher concentration, neutralized with 6 M HCl, and the final volume adjusted with MH broth. Afterwards, 100 μL of each extract was added to a respective well in a microplate, and serial dilutions (20 mg/mL to 1.25 mg/mL) were made in the following wells, where 50 μL of MH broth had previously been placed. Finally, 50 μL of MH broth with an inoculum of 10^6 CFU/mL of each bacterium under study was added to all wells, except for the negative controls. For all extracts and microorganisms studied, two negative controls (extract dissolved in MH broth (20 mg/mL) and only MH broth) and a positive control (100 μL of MH broth with 5×10^5 CFU) were made. All microplates were incubated at 37 °C for 24 h. The MICs were regarded as the lowest concentrations that did not allow any visible growth when compared with those of the control sets. To determine the MBC, 10 μL of wells where there was no visible growth of bacteria was inoculated in MH agar and incubated at 37 °C for 24 h. The MBC was considered the lowest concentration at which there was no bacterial growth. All tests

were performed in duplicate and results reported as the lowest concentration consistently producing the observed effect.

2.9.2. Antifungal activity

The antifungal activity of the extracts was assessed by determining the MICs and MBCs against four environmental filamentous fungi, namely *Aspergillus niger*, *Penicillium expansum, Fusarium verticillioides*, and *Cladosporium* sp., as well as the yeast *Malassezia furfur*. For the MICs ′s, the procedures were based on those previously described [24]. Spores of each fungus were harvested from a 7-day-old pure culture in Sabouraud Dextrose Agar (SDA) at 25 °C by rinsing the surface of the agar plates with 2 mL of a TWS solution (0.85% NaCl plus 0.05% Tween 80) and gently scraping spores from the surface. Spores were then collected and counted in a Neubauer chamber. The fungal concentration for the broth microdilution method was adjusted to 10^5 spores/mL. Five serially diluted concentrations (in Sabouraud Dextrose broth) of each peanut skin extract (20 mg/mL – 1.25 mg/mL) were prepared, and all were added in duplicate to a 96-well microtiter plate. For the alkaline extract, the stock solution was adjusted to neutral pH prior to preparation of the serial dilutions. A positive and a negative control were used. Then, the plates were incubated at 25 °C for 48 h. Afterwards, 10 μL from each well was plated onto SDA. Cultivation was conducted at 25 °C for 3 days. Regarding *M. furfur,* the determination of the MIC values followed the reference method by the Clinical and Laboratory Standards Institute (CLSI) documents M27, fourth edition (CLSI, 2017). The MICs were regarded as the lowest concentrations that did not allow any visible growth when compared with those of the control sets. All tests were performed in duplicate.

2.10. Antibiofilm activity

Antibiofilm activity was evaluated as described by Costa et al. [27] with minor modifications. Briefly, extracts were dissolved in Luria-Bertani (LB) broth for *E. coli* or in Tryptic soy broth (TSB) supplemented with 10% (w/v) glucose (Sigma, St. Louis, MO, USA) at two different concentrations, namely one-half (½×) and one-fourth (¼×) of the MIC. These sub-inhibitory concentrations are commonly employed to evaluate their potential effects on biofilm formation and bacterial behaviour without fully inhibiting growth. When no MIC was observed, the three highest concentrations (20, 10, and 5 mg/mL) were evaluated. Antibiofilm activity was evaluated against *E. coli, P. aeruginosa, S. aureus,* and *S. epidermidis*. Test solutions were inoculated with 2% (v/v) of an overnight culture (approximately 10^8 CFU/mL), transferred to a 96-well microtiter plate, and incubated for 24 h at 37 °C. The plate contents were then discarded, and each well was washed three times with sterile distilled water to remove non-adherent cells. The adhered cells were then fixed with 100 μL/well of methanol for 15 min. Then, the methanol was discarded, the plates were left to dry, and the fixed biofilm was stained with 105 μL/well of crystal violet (CV) for 5 min. Excess stain was removed under slow-running tap water. The plates were then air-dried, and bound dye was resolubilized with 105 μL/well of 33% (v/v) glacial acetic acid. Optical density was measured at 570 nm using a microtiter plate reader (Synergy H1). Assays were performed in triplicate with inoculated culture media as the positive control and sterile media as the negative control. The results were expressed as biomass inhibition percentages and calculated according to the following formula, in which OD assay is the OD measured in the presence of the extract, and OD positive control is the OD measured for the positive control.

$$\%\text{biomass formation inhibition} = 100 - \left(\frac{OD_{assay}}{OD_{positive\ control}}\right) \times 100$$

2.11. Cell based assays

Human keratinocytes (HaCaT, CLS 300493, Eppelheim, Germany) were selected as a representative in vitro skin model due to the intended focus of this study on skin-related and dermatological applications of peanut skin extracts. Cells were cultured as monolayers at 37 °C in a humidified atmosphere of 95% air and 5% CO_2, using DMEM with 4.5 g/L glucose, L-glutamine without pyruvate (ThermoScientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Lonza, Switzerland) and 1% (v/v) Penicillin-Streptomycin-Fungizone (ThermoScientific, Waltham, MA, USA). Keratinocytes were used between passages 53 and 58.

2.11.1. Cytotoxicity

The impact of the samples on HaCaT cell viability was performed according to the ISO 10993–5:2009 standard, as previously described by Costa et al. [28]. Cells were cultured to 80%−90% confluence, detached, and seeded at 1×10^5 cells/mL in a 96-well microplate. At 24 h, the medium was removed and replaced with fresh medium supplemented with test samples at concentrations varying from 500 μg/mL to 15.63 μg/mL. Plain medium was used as growth control, while medium supplemented with 30% (v/v) dimethyl sulfoxide (DMSO) was used exclusively as a death control. After 24 h of incubation, 10 μL of PrestoBlue (ThermoFisher, Waltham, MA, USA) was added to each well and incubated for 1 h. PrestoBlue is a resazurin-based assay in which metabolically active cells reduce the reagent to a fluorescent product, with fluorescence intensity being directly proportional to cellular metabolic activity and, consequently, cell viability. Afterwards, fluorescence was measured using an excitation wavelength of 560 nm with emission detected at 590 nm using a microplate reader (Synergy H1). All assays were conducted in quadruplicate, and the results were expressed as percentage inhibition of cell metabolism.

2.11.2. Cellular antioxidant activity

Cellular antioxidant activity was performed as previously described by Machado et al. [29]. Briefly, HaCaT cells were seeded at 2.5×10^5 cells/mL in a black 96-well microplate (Nunc Nunclon, ThermoScientific) and grown until confluence. After 24 h, the cells were washed with PBS, and Dichloro-dihydro-fluorescein diacetate (DCFH-DA) was added to the wells. Following incubation at 37 °C for 60 min, cells were washed, and test samples at the selected concentrations were added to the wells. As controls, plain medium (basal) or a free radical initiator (positive control for iROS production) was also added to the wells. The plate was incubated again, and subsequently, fluorescence was measured using an excitation wavelength of 480 nm with emission detected at 530 nm using a microplate reader (Synergy H1). All assays were performed in octuplicate, and results were expressed as a percentage of ROS species relative to the basal control.

2.12. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8.0.2 (GraphPad Software, La Jolla, CA, USA). The mean values and standard deviation (SD) within samples were calculated for all cases. Results were analyzed using One-way ANOVA followed by Tukey's post hoc test, with differences being considered significant for p-values < 0.05.

3. Results and discussion

3.1. Extraction yield and chemical composition

The results for the extraction yield, protein content, and total carbohydrate content are presented in Table 1. The highest yield was obtained with the alkaline methodology (23.98%), followed by both ultrasound extractions, hydroethanolic extraction, and finally aqueous extraction. Total protein content was highest in the aqueous extraction (14.92 g/100 g DE), while the lowest protein content was observed in ultrasound-assisted extraction (UAE#1) at 3.46 g/100 g DE. Regarding

Table 1
Extraction yield, protein and total carbohydrates content of peanut skin extracts. The results are presented as mean ± SD. Different letters in each column stand for statistical differences using a p-value < 0.05.

Peanut skin extract	Extraction yield (%)	Protein (g/100 g DE)	Total carbohydrates (mg GE/g DE)
NaOH	23.98	9.63 ± 0.14^{a}	308.98 ± 6.67^{a}
H_2O	12.31	14.92 ± 0.61^{b}	362.08 ± 24.07^{b}
EtOH	15.77	5.66 ± 0.54^{c}	538.75 ± 19.45^{c}
UAE#1	19.54	3.46 ± 0.27^{d}	397.65 ± 23.86^{b}
UAE#2	20.03	4.89 ± 0.34^{c}	359.83 ± 15.25^{b}

total carbohydrates, the hydroethanolic extract had the highest content (538.75 mg GE/g DE), whereas the alkaline extraction resulted in the lowest (308.98 mg GE/g DE).

Nepote et al. [7] reported similar extraction yields for ethanolic (100% v/v) and aqueous extracts, with yields of 18.5% and 9.9%, respectively. In the same study, the authors reported that the protein content of peanut skin was 12.32%. Braga et al. [30] also reported a yield of 15.17% for an extract obtained using 80% ethanol at a ratio of 1:10 (m/v). In another study, conducted by Mostafa et al. [31], the authors reported a protein content of 5.59% and a total carbohydrate content of 55.33% for an aqueous extract of peanut skin. Crude extracts obtained from blanching (BCE) and roasting (RCE) of peanut skins showed yields of 17.99% and 16.73%, respectively [5]. These crude extracts were obtained by defatting peanut skins with *n*-hexane for 6 h, followed by an ethanol–water (70:30 v/v) extraction for 24 h. These yield results are comparable with the ones reported in the present study and show that different yields of extract can be obtained using different extraction conditions. Another study, employing a microwave-assisted extraction methodology with a liquor ratio of 1:30 for 30 min using various solvents, i.e., water, ethanol, and hexane, reported yields of 7.9%, 7.3% and 4.8%, respectively [14]. These yields are, in turn, lower than the ones reported for the present extraction methodologies.

Regarding the TPC (Table 2), all extracts showed high amounts of phenolic content, however, the ultrasound assisted extracts yielded the highest values (512.93 mg GAE/g DE and 480.93 mg GAE/g DE), with no significant differences among the two conditions ($p > 0.05$). Based on the TPC result of UAE#1, we hypothesised that milling peanut skin (≤ 0.5 mm, UAE#2) could improve the extraction of phenolic compounds, resulting in a higher yield, and proceeded with its characterization as well. However, as the results show, this was not the case. The hydroethanolic extraction yielded the next best TPC with 313.10 mg GAE/g DE, followed by the alkaline extraction (264.42 mg GAE/g DE), with the aqueous extract yielding the lowest TPC content (120.47 mg GAE/g DE). In terms of total flavonoid content, the results followed the same pattern as for TPC, with the ultrasound extracts yielding the highest TFC (287.80 mg GAE/g DE and 264.35 mg CAE/g DE for UAE#1 and UAE#2, respectively) and the aqueous extract the lowest (68.78 mg CAE/g DE). The results showed that for all extractions, the TFC was more than 50% of all TPC, revealing the high quantity of flavonoids in these extracts. In the case of the alkaline extract, the TFC accounted for 72% of the TPC. Other studies have reported similar phenolic content, for instance, [30] reported (404.40 ± 13.22) mg GAE/g in an extract obtained with 80% ethanol. However, in this same study, the flavonoid content was lower, as the authors reported (16.14 ± 1.71) mg quercetin equivalent (QE)/g. The differences in flavonoid content might be related to the peanut cultivar, growth conditions or the methodology used for evaluating TFC [11]. Unfortunately, the aluminium chloride colorimetric assay suffers from several flaws. For instance, variety in the standards used might influence the results as different flavonoids have different chemical structures and consequently different absorption spectra [32]. Another hydroethanolic (50% v/v) extract from defatted peanut skins showed a TPC of 97 mg GAE/g and a flavonoid content of 65 mg CAE/g [33]. Yu et al. [34] reported that extraction solvents and skin removal methods (such as direct peeling, blanching, and roasting) had significant effects on total extractable phenolics. In this study, direct peeling with 80% (v/v) ethanol extraction yielded higher phenolics than water extraction (89.9 ± 2.20 mg GAE/g vs. 56.7 ± 0.54 mg GAE/g). This variety of results emphasizes the difference in the TPC using different extraction methodologies. Comparing with the literature, the UAE#1 methodology from the present paper stands out in terms of TPC and TFC.

Table 2
Content of total phenolics and flavonoids, and antioxidant activity (ORAC) of peanut skin extracts. The results are presented as mean ± SD. Different letters in each column stand for statistical differences using a p-value < 0.05.

Peanut skin extract	TPC (mg GAE/g DE)	TFC (mg CAE/g DE)	ORAC (μmol TE/g DE)
NaOH	264.42 ± 36.50^{a}	190.70 ± 0.99^{a}	1889.94 ± 270.37^{a}
H_2O	120.47 ± 3.88^{b}	68.78 ± 3.70^{b}	983.04 ± 42.20^{b}
EtOH	313.10 ± 6.66^{c}	166.65 ± 9.05^{c}	2210.06 ± 173.29^{a}
UAE#1	512.93 ± 29.24^{d}	287.80 ± 9.62^{d}	2770.17 ± 33.07^{c}
UAE#2	480.93 ± 25.82^{d}	264.35 ± 12.52^{e}	2925.00 ± 228.65^{c}

The antioxidant activity of the extracts was determined by the ORAC methodology. The results for antioxidant activity follow the same trend observed for TPC and TFC. The ultrasound-assisted extracts showed the best antioxidant activity, with no significant differences among them ((2770.17 ± 33.07) μmol TE/g DE and (2925.00 ± 228.65) μmol TE/g DE). The hydroethanolic extract showed the next highest antioxidant activity, followed by the alkaline extract and finally the aqueous extract, with an antioxidant activity of (983.04 ± 42.20) μmol TE/g DE. Other studies found in the literature used different methodologies and units to quantify antioxidant activity, making direct comparisons of the results more difficult. Nonetheless, several studies report the antioxidant potential of peanut skin extracts. The different methodologies used in other works include the 2,2-diphenyl−1-picrylhydrazyl-hydrate (DPPH) and 2,2'-azino-bis-(3-ethylbenz-thiazoline−6-sulfonic acid) (ABTS) assays. Braga et al. [30] reported DPPH and ABTS values of (756.54 ± 65.45) mg TE/g and (2.51 ± 0.44) g TE/g, respectively. Mostafa et al. [35] reported that the DPPH radical scavenging activity of peanut skin extract reached 63.30%, reinforcing its strong antioxidant capacity. On the other hand, Wang et al. [33] found that the water-soluble extracts from defatted peanut skins had 97.1% DPPH radical scavenging activity, strong scavenging activities against superoxide anions (98.6%), hydrogen peroxide (89.1%), and hydroxyl radicals (85.3%). Yu et al. [34] investigated the impact of different processing methods and solvents on peanut skin extracts. The antioxidant activity was measured as Trolox Equivalent Antioxidant Capacity (TEAC) and showed values of (4.10 ± 0.43) mM TE/mM phenolics for ethanol extracts and (3.39 ± 0.25) mM TE/mM phenolics for water extracts. These findings highlight the strong antioxidant potential of peanut skin extracts, particularly when extracted using ethanol. In the case of the UAE extracts obtained in this study, the antioxidant activity showed promising results and demonstrates significant potential as natural antioxidants in different industrial applications.

3.2. *Phenolic profile by LC-ESI-QqTOF-HRMS*

The phenolic profile of the different peanut skin extracts was obtained by LC-ESI-QqTOF-HRMS (Table 3). The peaks identified were grouped according to the mass (m/z) of corresponding deprotonated molecules. A wide range of phenolic compounds was detected, all of which were tentatively identified based on accurate mass measurements, fragmentation patterns, and comparison with literature data. Furthermore, the LC-ESI-QqTOF-HRMS data provided relative abundances based on peak area measurements and therefore represent a semi-quantitative assessment of the phenolic profile rather than absolute quantification.

Among the identified compounds, the peaks at $m/z = 153$ and 137 were particularly abundant in the alkaline extract, where they

Table 3
LC-ESI-UHR-QqTOF-MS data of phenolic compounds in different extracts from peanut skin extracts.

Peak	RT (min)	*m/z* calc. [M-H]⁻	MS2 [M-H]⁻	Proposed Formula	Tentative identification	Extracts; Peak area – a.u. (Relative abundance - %)				
						NaOH	H_2O	EtOH	UAE#1	UAE#2
1	1.8	191.0197	85.0296, **87.0108** *, 111.0091	$C_6H_8O_7$	Citric acid	2.76×10^6 (2.01)	6.54×10^6 (6.00)	n.d.	7.97×10^6 (1.11)	9.72×10^6 (1.75)
3	2.8	403.1245	**109.0296**, 113.0254, 271.0827	$C_{17}H_{24}O_{11}$	Phenolic O-pentoside	n.d.	2.80×10^6 (2.57)	4.74×10^6 (1.14)	3.11×10^6 (0.43)	3.33×10^6 (0.60)
4	4.2	167.0350	93.0346, **108.0217**	$C_8H_8O_4$	Phenolic acid isomer	n.d.	3.25×10^6 (2.98)	5.56×10^6 (1.34)	6.95×10^6 (0.97)	6.56×10^6 (1.18)
5	5.0	153.0195	91.0186, **108.0217**, 109.0262	$C_7H_6O_4$	Gentisic acid (2,5-dihydroxybenzoic acid)	2.29×10^7 (16.74)	5.02×10^6 (4.60)	1.06×10^7 (2.55)	1.37×10^7 (1.91)	1.52×10^7 (2.74)
6	8.1	137.0244	92.0268, **102.0211**	$C_7H_6O_3$	3,4-Dihydroxybenzaldehyde	6.47×10^7 (47.25)	2.37×10^7 (21.71)	4.68×10^7 (11.31)	5.39×10^7 (7.48)	5.57×10^7 (10.05)
7	11.1	577.1351	125.0246, 245.0247, **289.0822**, 339.0878, 407.0774	$C_{30}H_{26}O_{12}$	Procyanidin dimer B-type	n.d.	n.d.	2.85×10^7 (6.88)	5.43×10^7 (7.53)	4.74×10^7 (8.56)
		163.0403	93.0348, **119.0507**	$C_9H_8O_3$	*p*-Coumaric acid	n.d.	2.41×10^7 (22.06)	2.03×10^7 (4.90)	n.d.	n.d.
8	12.3	289.0718	97.0295, 109.0295, **123.0452**, 125.0244, 135.0452, 151.0401, 188.0479, 203.0714, 205.0506, 221.0819	$C_{15}H_{14}O_6$	Catechin	n.d.	9.29×10^6 (8.52)	1.90×10^7 (4.58)	4.66×10^7 (6.47)	3.69×10^7 (6.66)
9	13.1	431.1520	89.0244, 101.0244, **113.0244**, 119.0350, 119.0502, 137.0608, 149.0455, 161.0455, 191.0561, 299.1136	$C_{19}H_{28}O_{11}$	Phenolic O-pentoside	n.d.	5.77×10^6 (5.29)	8.32×10^6 (2.01)	n.d.	n.d.
11	14.7	577.1357	125.0246, 245.0249, **289.0719**, 339.0874, 407.0775	$C_{30}H_{26}O_{12}$	Procyanidin dimer B-type	n.d.	n.d.	7.99×10^6 (1.93)	1.53×10^7 (2.12)	1.21×10^7 (2.18)
12	16.3	575.1195	125.0225, 151.0385, **289.0686**, 411.0687	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	n.d.	1.43×10^7 (3.45)	2.83×10^7 (3.92)	2.39×10^7 (4.32)
13	17.2	863.1833	125.0244, 289.0718, 407.0772, 423.0722, 539.0984, 559.0882, **575.1195**, 693.1250	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	1.07×10^7 (2.59)	2.45×10^7 (3.41)	1.47×10^7 (2.65)
14	18.3	289.0723	97.0270, 109.0284, **123.0432**, 151.0367, 221.0796	$C_{15}H_{14}O_6$	Catechin	n.d.	n.d.	5.37×10^6 (1.30)	1.10×10^7 (1.52)	9.99×10^6 (1.80)
15	18.9	167.0350	**108,0217**	$C_8H_8O_4$	Phenolic acid isomer	6.96×10^6 (5.08)	n.d.	n.d.	n.d.	n.d.
16	19.0	863.1829	125.0244, **289.0718**, 299.0561, 407.0772, 411.0722, 451.1035, 559.0882, 693.125	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	7.43×10^6 (1.79)	1.55×10^7 (2.15)	1.07×10^7 (1.92)
19	20.3	863.1794	125.0244, **289.0718**,	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	8.29×10^6 (2.00)	1.21×10^7 (1.68)	9.85×10^6 (1.78)

(*continued on next page*)

Table 3 (*continued*)

Peak	RT (min)	*m/z* calc. [M-H]⁻	MS2 [M-H]⁻	Proposed Formula	Tentative identification	Extracts; Peak area – a.u. (Relative abundance - %)				
						NaOH	H_2O	EtOH	UAE#1	UAE#2
			299.0561, 407.0772, 411.0722, 451.1035, 559.0882, 693.1250							
20	21.9	607.1283	112.988, 119.0502, **163.0401**, 167.035, 203.035, 209.0455, 277.0354	$C_{27}H_{28}O_{16}$	Hydroxycinnamate-containing phenolic conjugate	n.d.	3.48×10^6 (3.19)	3.24×10^6 (0.78)	n.d.	n.d.
22	22.6	577.1351	125.0246, 151.0385, 161.0244, 245.0247, **289.0822**, 339.0878, 407.0774, 411.0722	$C_{30}H_{26}O_{12}$	Procyanidin dimer B-type	n.d.	n.d.	3.92×10^6 (0.95)	1.06×10^7 (1.47)	6.82×10^6 (1.23)
23	24.1	575.1198	125.0247, 245.0824, **285.0408**, 289.0720, 327.0510, 407.0774, 449.0868, 539.0987	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	2.02×10^7 (18.56)	1.04×10^8 (25.15)	1.79×10^8 (24.83)	1.36×10^8 (24.53)
24	24.7	575.1198	125.0244, 151.0401, 245.0819, **285.0405**, 289.0718, 327.0510, 407.0772, 423.0722, 539.0984	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	n.d.	9.32×10^6 (2.25)	n.d.	1.33×10^7 (2.40)
25	25.0	863.1829	125.0244, 289.0718, 407.0772, 423.0722, 539.0984, **575.1195**, 693.125, 711.1355	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	4.31×10^7 (10.39)	1.08×10^8 (14.99)	5.64×10^7 (10.18)
26	27.5	863.1823	125.0244, 285.0384, 289.0718, 407.0772, **411.0695**, 451.1008, 559.0854, 693.1250	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	n.d.	1.11×10^7 (1.54)	5.60×10^6 (1.01)
29	28.4	575.1195	125.0244, 151.0388, **285.0384**, 289.0718, 407.0757, 411.0698, 449.0855, 539.0963	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	n.d.	1.02×10^7 (2.46)	2.27×10^7 (3.15)	1.13×10^7 (2.03)
30	28.7	575.1195	125.0244, 245.0819, **285.0405**, 289.0718, 327.0510, 407.0772, 423.0722, 452.0749, 539.0984	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	2.51×10^6 (2.30)	2.08×10^7 (5.01)	3.91×10^7 (5.43)	1.89×10^7 (3.41)
31	29.8	181.0509	**108.0219**, 109.0300	$C_9H_{10}O_4$	Hydroxybenzoic acid derivative	6.42×10^6 (4.68)	n.d.	n.d.	n.d.	n.d.
32	29.9	575.1168	125.0247, **289.0405**,	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	4.48×10^6 (3.27)	n.d.	n.d.	n.d.	n.d.

(*continued on next page*)

Table 3 (*continued*)

Peak	RT (min)	*m/z* calc. [M-H]⁻	MS2 [M-H]⁻	Proposed Formula	Tentative identification	Extracts; Peak area – a.u. (Relative abundance - %)				
						NaOH	H_2O	EtOH	UAE#1	UAE#2
			327.0510, 407.0775, 423.0720, 449.0874, 539.0988							
33	30.1	863.1829	125.0244, 161.0245, 289.0718, 407.0772, 423.0722, 539.0984, **575.1195**, 693.125	$C_{45}H_{36}O_{18}$	Procyanidin trimer A-type	n.d.	n.d.	n.d.	7.69×10^6 (1.07)	3.41×10^6 (0.62)
34	30.4	1149.2279	285.0390, 411.0703, 449.0856, **575.1175**, 979.1705	$C_{60}H_{46}O_{24}$	Procyanidin tetramer 2 A-type linkages	n.d.	n.d.	n.d.	1.63×10^7 (2.26)	n.d.
35	30.6	575.1168	125.0244, 151.0388, **285.0384**, 289.0718, 407.0757, 411.0698, 449.0855, 539.0963	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	2.87×10^7 (20.96)	n.d.	6.91×10^6 (1.67)	1.02×10^7 (1.45)	1.85×10^7 (3.33)
37	31.1	623.1623	**315,0513**	$C_{28}H_{32}O_{16}$	Isorhamnetin rutinoside	n.d.	2.41×10^6 (2.21)	4.86×10^6 (1.17)	3.60×10^6 (0.51)	4.79×10^6 (0.86)
39	31.3	575.1192	125.0244, 151.0401, 161.0244, **285.0405**, 289.0718, 407.0772, 411.0722, 539.0984	$C_{30}H_{24}O_{12}$	Procyanidin dimer A-type	n.d.	n.d.	4.98×10^6 (1.20)	5.55×10^6 (0.79)	1.41×10^7 (2.55)
40	31.6	573.1022	125.0244, 151.0388, **285.0384**, 289.0718, 411.0698, 449.0855	$C_{30}H_{22}O_{12}$	Procyanidin dimer A-type	n.d.	n.d.	n.d.	2.75×10^6 (0.39)	3.77×10^6 (0.68)
48	35.9	301.0354	121.0295, **151.0037**, 178.9986	$C_{15}H_{10}O_7$	Quercetin	n.d.	n.d.	4.95×10^6 (1.19)	1.08×10^7 (1.54)	5.26×10^6 (0.95)
					Total Phenolic Area (a. u.)	1.37×10^8	1.09×10^8	4.14×10^8	7.21×10^8	5.44×10^8

* **Bold** represents the main fragment; [M-H]- is deprotonated molecular ion.

accounted for 16.74% and 47.25% of the total phenolic peak area, respectively. The compound at $m/z = 153$ was tentatively identified as gentisic acid while the peak at $m/z = 137$ was assigned to 3,4-dihydroxybenzaldehyde. These m/z values have also been reported in previous studies on peanut skin extracts, where they were among the most abundant low molecular weight phenolics [36]. One peak was only detected in the alkaline extraction, peak at $m/z = 181$, tentatively identified as a phenolic acid derivative. The distinct phenolic profile observed for the alkaline extract suggests that the alkaline extraction conditions promoted structural modifications of native procyanidins, including oxidation, rearrangement reactions, and cleavage of interflavan linkages, as previously reported under alkaline treatment conditions [17]. These modifications may explain the reduced abundance of procyanidin oligomers, and the higher presence of low molecular weight phenolic compounds detected in this extract.

Regarding the H_2O extract, the main peaks were $m/z = 137$ (21.71%) and 163 (22.06%). The latter one was tentatively identified as *p*-coumaric acid due to the fragmentation ions at m/z 93 and 119.

The hydroethanolic and ultrasound-assisted extracts (UAE#1 and UAE#2) revealed similar phenolic profiles with the main peaks detected at $m/z = 289$, 575, 577, and 863. Based on the molecular structure and mass, these peaks were tentatively identified as catechin/epicatechin monomers (MW = 290), A-type procyanidin dimers (MW = 576), B-type procyanidin dimers (MW = 578), and A-type procyanidin trimers (MW = 864), respectively. Among these, A-type procyanidin dimers were the predominant compounds in these three extracts, with relative percentages of 39.99%, 38.78% and 40.02% for the hydroethanolic, UAE#1, and UAE#2 extracts, respectively. These results are consistent with previous studies where proanthocyanidins were the main phenolic compounds reported [5,8,12,36]. For instance, de Camargo et al. [8] reported that in a 70% (v/v) acetone extraction followed by vacuum concentration, procyanidins accounted for 84% of the phenolics, whereas monomeric flavonoids and phenolic acids accounted for 13% and 3%, respectively. The same was reported in a 70% (v/v) hydroethanolic extraction at 80 °C, where the main phenolic compounds detected were procyanidin monomers, A-type and B-type procyanidin dimers and A-type procyanidin trimers [36].

Several procyanidin oligomers with identical m/z values were detected at different retention times, indicating the presence of multiple structural isomers, as previously reported [36]. This structural diversity reflects variations in interflavan linkages and degrees of polymerization. Consistent with Monagas et al. [37], the proanthocyanidins content in a

peanut skin extract was substantially higher than that of monomeric flavonoids.

The last peak, at $m/z = 301$, was detected in the hydroethanolic and UAE extracts and was tentatively identified as quercetin due to the fragmentation pattern, with ions at m/z 121, 151 and 179. Although quercetin is a well-known bioactive flavonol, its relative abundance in the extracts was low (approximately 1%), in agreement with previous reports on peanut skin phenolic composition [5,12]. Additionally, the presence of flavonoid glycosides such as isorhamnetin rutinoside further indicates that peanut skins contain a diverse range of flavonoid subclasses beyond flavan−3-ols,

Regarding the relative quantification of phenolic compounds (expressed as peak are, a.u.), the LC-ESI-QqTOF-HRMS results followed the same trend observed by the TPC quantification, with the aqueous extract presenting the lowest phenolic levels, followed by the alkaline, hydroethanolic, UAE#2 and finally, UAE#1. Overall, these results highlight that, although proanthocyanidins are the dominant phenolic compounds in peanut skins, the matrix contains a chemically diverse array of phenolics, including simple phenolic acids, aldehydes, flavonoids, and complex conjugates. Among the extraction methods evaluated, ultrasound assisted extraction #1 proved to be the most effective for maximizing phenolic compound recovery while maintaining a broad and representative phenolic profile.

3.3. Antimicrobial activity

Regarding the results of antimicrobial activity of the peanut skin extracts, Table 4 shows the MICs of the extracts against the tested bacteria and fungi. From these results, it is possible to observe that the alkaline extract provided the best antimicrobial activity, inhibiting the growth of all bacteria tested, although at different concentrations. It was able to inhibit the Gram-negative bacteria *P. aeruginosa* at 10 mg/mL and *E. coli* at 20 mg/mL, while inhibiting the Gram-positive bacteria *S. aureus* and *S. epidermidis* at lower concentrations, namely 5 mg/mL and 2.5 mg/mL, respectively. The enhanced antimicrobial activity of the alkaline extract may be related, at least in part, to the compositional changes induced by the alkaline extraction, which resulted in a phenolic profile enriched in low molecular weight phenolic constituents, such as gentisic acid and other phenolic acid derivatives. These compounds have been reported to exert antimicrobial effects and may contribute additively or synergistically to the overall antimicrobial activity of the extract [38]. The hydroethanolic and ultrasound-assisted extracts were able to inhibit both Gram-positive bacteria at the highest tested concentration of 20 mg/mL. In contrast, the aqueous extract exhibited no antimicrobial activity against the tested bacteria. In addition, none of the peanut skin extracts obtained here were able to inhibit the growth of filamentous fungi at the highest tested concentration of 20 mg/mL. In the case of *M. furfur*, the alkaline extract, along with both UAE extracts, demonstrated notable antifungal activity. The alkaline extract exhibited the highest potency, with a MIC below 1.25 mg/mL, while both ultrasound-assisted extracts had MICs of 2.5 mg/mL. In contrast, the hydroethanolic extract showed no activity against *M. furfur*, and the aqueous extract displayed weaker activity, with a MIC of 10 mg/mL. This finding is particularly relevant given that *M. furfur* is a dominant component of the human skin mycobiota and plays a key role in the development and exacerbation of common dermatological disorders, including dandruff, seborrheic dermatitis, pityriasis versicolor, and folliculitis [39,40]. The observed antifungal activity against *M. furfur* therefore supports the potential applicability of peanut skin extracts in skin-related formulations aimed at controlling yeast overgrowth while aligning with the demand for natural and sustainable bioactive ingredients.

The antimicrobial activity of peanut skin extracts has been investigated across different studies. However, other extraction methods were used, and only a few of them describe MIC or MBC values. For instance, a 70% (v/v) acetone showed MIC values of 301 μg/mL against *Bacillus cereus, S. aureus, P. aeruginosa, Pseudomonas fluorescens, Salmonella* Enteritidis, and *Salmonella* Typhimurium, and 602 μg/mL against *Listeria monocytogenes* and *E. coli* [8]. These values are significantly lower than the ones obtained in the present study. An extract obtained from repeated boiling water extractions followed by vacuum concentration showed the lowest MIC values against *B. cereus* (0.25 mg/mL) and MICs $>$ 5.05 mg/mL for other bacteria, including *S. aureus* and *E. coli* [41]. Activity against yeast and filamentous fungi has also been reported for peanut skin extracts. Sarnoski et al. [42] examined the antifungal properties of peanut skin proanthocyanidins against *Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Zygosaccharomyces bisporus*. The study found that at 1 mg/mL, peanut skin extract extended the lag phase of yeast growth, while at 10 mg/mL, yeast growth was completely inhibited for 120 h.

These studies show that peanut skin extracts appear to be more effective against Gram-positive bacteria than Gram-negative bacteria [8,41]. They also highlight that the most active compounds are A-type proanthocyanidin dimers, trimers, and tetramers and that peanut skin extracts exert antimicrobial effects through cell membrane disruption, metabolic interference, and inhibition of glucose uptake [8,41,42]. Regarding filamentous fungi, fractions obtained from a double extraction with 70% (v/v) ethanol, followed by evaporation and fractionation, revealed a significant reduction in *F. verticillioides* radial growth in the range of 250 μg/mL – 500 μg/mL [43].

It should be noted that the present study evaluated crude peanut skin extracts rather than purified or enriched fractions, which may partly explain the higher MIC values observed compared with some literature reports. Nevertheless, for topical applications such as cosmetic formulations or functional textile finishes, concentrations in the mg/mL range may remain feasible. From a bioprocess perspective, these findings highlight the importance of selecting extraction methodologies according to the intended application, as different extraction conditions

Table 4
Antimicrobial activity of the different peanut skin extracts.

Microorganisms	**MIC and MBC (mg/mL)**									
Bacteria	**NaOH**		**H_2O**		**EtOH**		**UAE#1**		**UAE#2**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	20	20	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
P. aeruginosa	10	10	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
S. aureus	5	5	n.i	n.i	20	20	20	20	20	20
S. epidermidis	2.5	5	n.i	n.i	20	20	20	20	20	20
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. niger	n.i.	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
Cladospotium sp.	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
F. verticillioides	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
P. expansum	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i	n.i
M. furfur	$<$ 1.25	2.5	10	10	n.i.	n.i.	2.5	2.5	2.5	2.5

n.i. – No inhibition observed at the highest concentration of 20 mg/mL.

generate extracts with distinct phenolic profiles and biological properties. Further purification, enrichment of active phenolic fractions, or formulation optimization could enhance antimicrobial efficacy and further reduce the concentrations required for practical applications, supporting the development of sustainable peanut skin valorisation strategies.

3.4. Antibiofilm activity

The results regarding antibiofilm activity (Fig. 1) indicate that peanut skin extracts effectively reduced the biofilm formation capacity of the Gram-positive bacteria at sub-inhibitory concentrations. For instance, all extracts at one-half MIC and one-fourth MIC were able to inhibit *S. aureus* biofilm formation by more than 80%. Regarding *S. epidermidis*, the EtOH, UAE#1 and UAE#2 extracts also inhibited biofilm formation by over 80%. The aqueous extract inhibited around 80% of biofilm formation while the alkaline extract had the lowest antibiofilm activity (below 80%).

Regarding the Gram-negative bacteria, when the bacterial solution was added to each microplate well containing the different peanut skin extracts, the extracts precipitated in varying amounts (depending on the extract and concentration). These precipitates did not wash away even after the washing steps of the antibiofilm protocol and increased in the presence of both bacteria. For this reason, the results for both Gram-negative bacteria are not shown.

Since biofilm formation was evaluated at sub-inhibitory concentrations (one-half MIC and one-fourth MIC), the observed biofilm inhibition effects are unlikely to be associated with bacterial growth inhibition. Instead, the results suggest that peanut skin extracts may interfere with bacterial adhesion and early biofilm development. This interpretation is consistent with high inhibition percentages observed even at concentrations below those required to inhibit planktonic growth.

No previous studies have reported the antibiofilm activity of peanut skin extracts. However, considering their main compounds, such as procyanidins, previous research supports their antibiofilm potential, aligning with our findings. Procyanidins have been shown to inhibit bacterial adhesion to food or equipment surfaces, as well as biofilm formation [44–48]. Additionally, A-type procyanidins have exhibited superior activity compared to B-type procyanidins in inhibiting bacterial adhesion and biofilm formation [49].

3.5. Cytotoxicity

The data obtained regarding the impact of peanut skin extracts towards HaCaT cell metabolism (Fig. 2) showed that the EtOH extract had the highest non-cytotoxic concentration (500 μg/mL) followed by the H_2O extract (250 μg/mL), NaOH (125 μg/mL) and UAE#1 and UAE#2 (62.50 μg/mL and 31.25 μg/mL, respectively). These were the concentrations below the 30% inhibition threshold defined as safe by the ISO Standard 10993–5. The higher cytotoxicity observed for the UAE extracts at lower concentrations can be associated with their phenolic composition and higher relative abundance of bioactive compounds, which may exert stronger biological effects on keratinocytes. As shown by the LC-ESI-QqTOF-HRMS analysis, UAE extracts were particularly rich in type-A and type-B procyanidin dimers and trimers, compounds known to exhibit strong redox activity and pronounced biological effects at the cellular level [50]. Ultrasound-assisted extraction is known to enhance cell wall disruption and mass transfer, resulting in extracts enriched in phenolic compounds with higher biological activity, as reflected by their increased antioxidant and antimicrobial effects. While these properties are desirable for functional applications, the higher reactivity of phenolic-rich extracts may also result in increased cytotoxic effects at lower doses, highlighting the importance of defining appropriate and safe concentration ranges for skin-related applications.

To the best of our knowledge, no previous work reports cytotoxic characterization of peanut skin extracts against HaCaT cell line. However, other studies have reported that a pressure assisted peanut skin hydroethanolic (40:60, v/v), with quercetin as the main compound, was not cytotoxic up to 300 μg/mL towards ileum epithelial cells (IEC−18) and Vero cells [51]. Although the antioxidant properties of peanut skin phenolics are well-known, there is still limited information regarding their potential cytotoxic effects.

3.6. Cellular antioxidant activity

Given the cytotoxicity results, the non-cytotoxic concentrations were chosen to evaluate the cellular antioxidant activity of the peanut skin extracts (Fig. 3). At these concentrations, peanut skin extracts did not exhibit any pro-oxidant potential, as no increase in intracellular ROS (iROS) production was observed. In fact, although the reduction was not statistically significant ($p > 0.05$), a slight decrease in iROS production was observed in the presence of all extracts compared to basal iROS levels.

This overall antioxidant behaviour can be associated with the

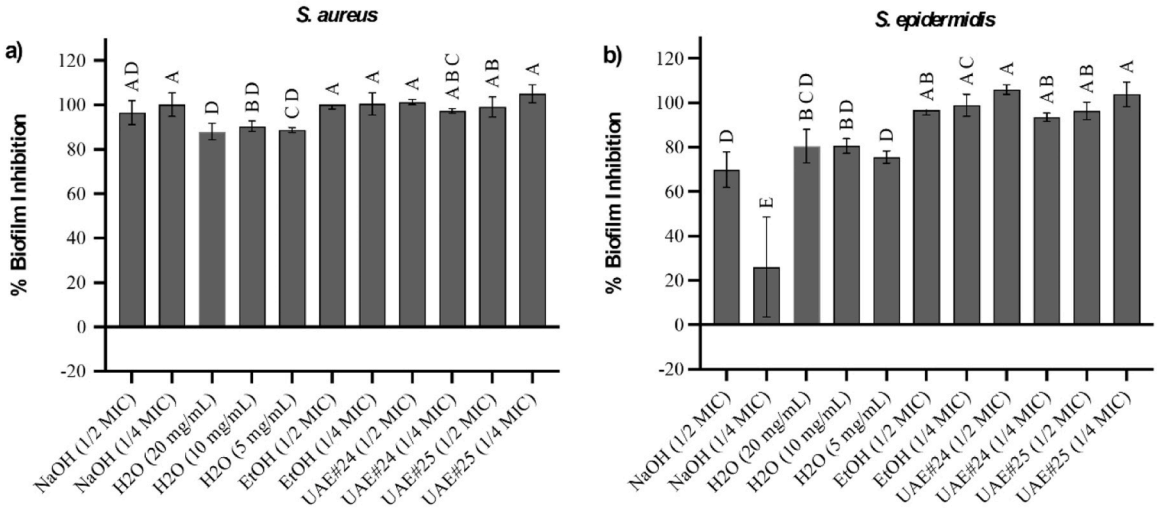

Fig. 1. Biofilm formation values for peanut skin extracts against *S. aureus* (a), *S. epidermidis* (b). Different letters above each bar stand for statistically significant differences ($p < 0.05$).

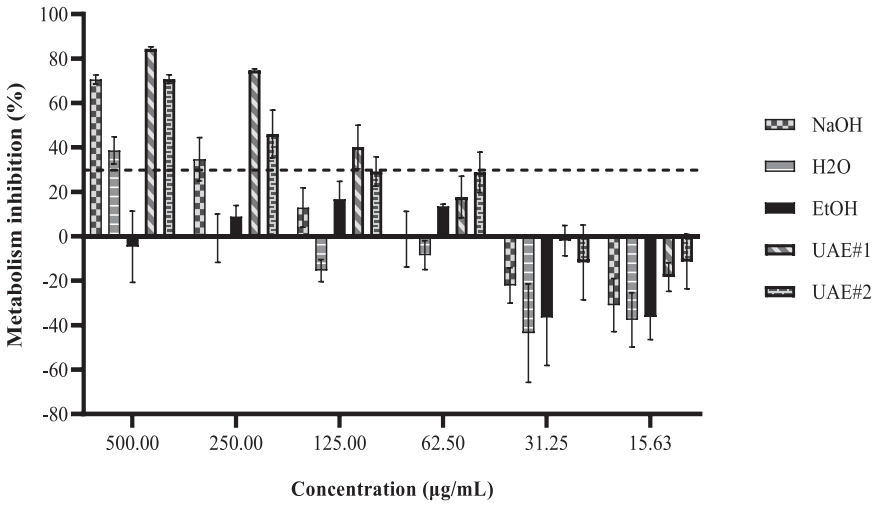

Fig. 2. Evaluation of the impact of peanut skin extracts on HaCaT cell metabolism.

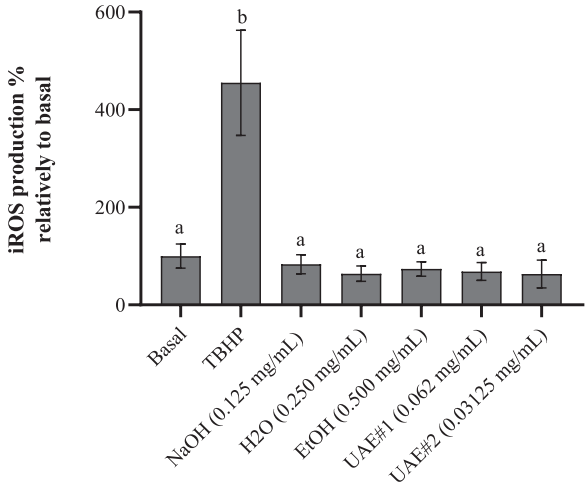

Fig. 3. Peanut skin extracts cellular antioxidant activity. Different letters above each bar represent statistical differences using a p-value < 0.05.

phenolic composition of the peanut skin extracts, dominated by type-A and type-B procyanidin dimers and trimers. The presence of these compounds is consistent with the antioxidant behaviour observed at the cellular level, supporting a protective effect against oxidative stress when applied within safe concentration ranges. The consistent antioxidant response observed across all extracts suggests that, despite quantitative differences in phenolic content, the shared presence of these compound classes plays a central role in the cellular antioxidant effects.

Skin is a primary target for the generation of ROS, and these reactive species play critical roles in the development of various diseases, including cancer, aging, atherosclerosis, and cardiovascular diseases [52]. Conversely, ROS are also involved in essential skin tissue regeneration processes, such as inflammation regulation, cell proliferation, angiogenesis and granulation [53]. The findings presented here underscore the potential of peanut skin extracts as safe and effective antioxidants for skin applications. Their ability to prevent excessive ROS accumulation without exhibiting pro-oxidant effects further supports their potential for use in skin products, therapeutic formulations, or as functional ingredients in skin-protective materials.

4. Conclusions

This study highlights the potential of peanut skin as a bioactive agro-industrial by-product, rich in phenolic compounds with potent antioxidant and antimicrobial properties. Among the extraction methods evaluated, UAE yielded the highest phenolic content, followed by hydroethanolic and alkaline extraction, while the aqueous extraction was the least effective. The extracts, particularly those obtained via UAE, contained high amounts of procyanidins, contributing to strong antioxidant activity, making them promising candidates in various industries, including food preservation, pharmaceuticals, and cosmetics. Additionally, the extracts exhibited antimicrobial effects, especially against Gram-positive bacteria, and effectively inhibited biofilm formation, suggesting their potential as natural preservatives and antimicrobial agents. Notably, despite the general lack of antifungal activity against filamentous fungi, selected extracts, particularly alkaline and ultrasound-assisted, demonstrated effective inhibition of *M. furfur*, highlighting a targeted antifungal potential. All extracts showed the capacity to reduce iROS formation in HaCaT cells. Taken together, these findings support the potential of peanut skin extracts for skin-related applications. The reference to textiles is presented as a prospective application, grounded in the observed antioxidant and antimicrobial properties of the extracts and supported by previous studies on bio-based textile finishing, rather than on direct textile testing. From this perspective, peanut skin extracts could contribute to sustainable textile finishing by providing additional functionalities such as UV protection, antimicrobial resistance, and natural coloration, in line with the growing demand for eco-friendly solutions. Overall, this work highlights the valorisation potential of peanut skin within a circular economy framework and demonstrates that extraction methodology is a key process variable influencing the recovery of phenolic compounds and the resulting biological properties of the extracts. These findings support the development of sustainable bioprocesses for the recovery of high-value bioactive ingredients from peanut skin. By integrating peanut skin-derived bioactive compounds into diverse industrial sectors, this study contributes to the development of environmentally friendly and high-value applications, reducing waste and enhancing resource efficiency.

Future studies should address the scalability of the most promising extraction methods, together with optimization of process parameters, to support industrial implementation. Additional work on extract standardization, stability, and application-oriented validation will be required to facilitate the translation of peanut skin-derived bioactive compounds into practical and sustainable products.

CRediT authorship contribution statement

Eduardo M. Costa: Writing – review & editing, Investigation, Data

curation. **Costa Filipa:** Project administration, Investigation. **Teresa Bonifácio-Lopes:** Writing – review & editing, Methodology, Investigation. **Tânia Ribeiro:** Writing – review & editing, Formal analysis. **Tiago Barros Afonso:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carla J. Silva:** Resources, Funding acquisition. **Manuela Pintado:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Joana Moreira:** Methodology, Investigation. **Oliveira Juliana A. S. A.:** Writing – review & editing, Project administration. **Daniel Mendanha:** Writing – review & editing, Project administration, Conceptualization. **Tiago Macedo:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that the work described in this manuscript has not been published previously and that it is not under consideration for publication elsewhere. Its publication has been approved by all authors and, where applicable, by the responsible authorities of the institutions where the work was carried out. If accepted, the article will not be published elsewhere in the same form, in English or any other language, including electronically, without the written consent of the copyright-holder.

The authors have no conflicts of interest to declare.

Acknowledgments

Integrated Project be@t – Textile Bioeconomy, to strengthen the National Bioeconomy, financed by the Environmental Fund through Component 12 – Promotion of Sustainable Bioeconomy (Investment TC-C12-i01 – Sustainable Bioeconomy No. 02/C12- i01.01/2022), of European funds allocated to Portugal by the Recovery and Resilience Plan (RRP), within the scope of the European Union (EU) Recovery and Resilience Mechanism, framed in the Next Generation EU, for the period 2021 – 2026. This work was also supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UID/50016/2025.

Data availability

Data will be made available on request.

References

[1] B.R. Albuquerque, S.A. 8. Heleno, M.B.P.P. Oliveira, L. Barros, I.C.F.R. Ferreira, Phenolic compounds: current industrial applications, limitations and future challenges, Food Funct. 12 (2021) 14–29, https://doi.org/10.1039/d0fo02324h.

[2] R. Mark, X. Lyu, J.J.L. Lee, R. Parra-Saldívar, W.N. Chen, Sustainable production of natural phenolics for functional food applications, J. Funct. Foods 57 (2019) 233–254, https://doi.org/10.1016/j.jff.2019.04.008.

[3] P. Brudzyńska, A. Sionkowska, M. Grisel, Plant-derived colorants for food, cosmetic and textile industries: a review, Materials 14 (2021), https://doi.org/10.3390/ma14133484.

[4] T.B. Afonso, T. Bonifácio-Lopes, E.M. Costa, M.E. Pintado, Phenolic compounds from by-products for functional textiles, Materials 16 (2023), https://doi.org/10.3390/ma16227248.

[5] M. Larrauri, M.P. Zunino, J.A. Zygadlo, N.R. Grosso, V. Nepote, Chemical characterization and antioxidant properties of fractions separated from extract of peanut skin derived from different industrial processes, Ind. Crops Prod. 94 (2016) 964–971, https://doi.org/10.1016/j.indcrop.2016.09.066.

[6] G. Barreto Costa, N.L. Hernandez Brito, K. Pinheiro Martins, J. Silva Ramos, A.C. S. Barbosa Alessi, T.A. Santos, A.E. Melo da Silva, A.A. Droval, F.A.R. Cardoso, Comprehensive valorization of peanut (Arachis hypogaea L.) fractions from seed to hull: compositional insights, bioactive potential, and sustainable applications, Food Rev. Int. (2026), https://doi.org/10.1080/87559129.2026.2639510.

[7] V. Nepote, N.R. Grosso, C.A. Guzman, Extraction of antioxidant components from peanut skins, Grasas Y. Aceites 53 (2002) 391–395, https://doi.org/10.3989/gya.2002.v53.i4.335.

[8] A.C. de Camargo, M.A.B. Regitano-d'Arce, G.B. Rasera, S.G. Canniatti-Brazaca, L. do Prado-Silva, V.O. Alvarenga, A.S. Sant'Ana, F. Shahidi, Phenolic acids and flavonoids of peanut by-products: antioxidant capacity and antimicrobial effects, Food Chem. 237 (2017) 538–544, https://doi.org/10.1016/j.foodchem.2017.05.046.

[9] Y. Chukwumah, L.T. Walker, M. Verghese, Peanut skin color: a biomarker for total polyphenolic content and antioxidative capacities of peanut cultivars, Int. J. Mol. Sci. 10 (2009) 4941–4952, https://doi.org/10.3390/ijms10114941.

[10] A.K. Redhead, N.F.I.N. Azman, A.I. Nasaruddin, T. Vu, F. Santos, R. Malheiros, A.S. M. Hussin, O.T. Toomer, Peanut skins as a natural antimicrobial feed additive to reduce the transmission of salmonella in poultry meat produced for human consumption, J. Food Prot. 85 (2022) 1479–1487, https://doi.org/10.4315/JFP-21-205.

[11] J.M. Lorenzo, P.E.S. Munekata, A.S. Sant'Ana, R.B. Carvalho, F.J. Barba, F. Toldrá, L. Mora, M.A. Trindade, Main characteristics of peanut skin and its role for the preservation of meat products, Trends Food Sci. Technol. 77 (2018) 1–10, https://doi.org/10.1016/j.tifs.2018.04.007.

[12] A.C. de Camargo, M.A.B. Regitano-d'Arce, C.R. Gallo, F. Shahidi, Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin, J. Funct. Foods 12 (2015) 129–143, https://doi.org/10.1016/j.jff.2014.10.034.

[13] M. Michalak, Plant extracts as skin care and therapeutic agents, Int. J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms242015444.

[14] M. Rehan, N.S. Elshemy, K. Haggag, A.S. Montaser, G.E. Ibrahim, Phytochemicals and volatile compounds of peanut red skin extract: simultaneous coloration and in situ synthesis of silver nanoparticles for multifunctional viscose fibers, Cellulose 27 (2020) 9893–9912, https://doi.org/10.1007/s10570-020-03452-8.

[15] D. Grifoni, L. Bacci, S. Di Lonardo, P. Pinelli, A. Scardigli, F. Camilli, F. Sabatini, G. Zipoli, A. Romani, UV protective properties of cotton and flax fabrics dyed with multifunctional plant extracts, Dyes Pigments 105 (2014) 89–96, https://doi.org/10.1016/j.dyepig.2014.01.027.

[16] M.M. Hassan, Enhanced insect-resistance, UV protection, and antibacterial and antistatic properties exhibited by wool fabric treated with polyphenols extracted from mango seed kernel and feijoa peel, RSC Adv. 11 (2021) 1482–1492, https://doi.org/10.1039/d0ra09699g.

[17] I.Bin Imran, M. Karonen, J.P. Salminen, M.T. Engström, Modification of natural proanthocyanidin oligomers and polymers via chemical oxidation under alkaline conditions, ACS Omega 6 (2021) 4726–4739, https://doi.org/10.1021/acsomega.0c05515.

[18] L. Yu, S. Feng, Y. Song, J. Bi, Y. Gao, L. Wang, C. Jiang, M. Wang, Exploring the extraction, antioxidant activities and its stabilities of peanut skins crude proanthocyanidins extract, Sci. Rep. 15 (2025), https://doi.org/10.1038/s41598-025-10934-x.

[19] S. Jin, M. Gao, W. Kong, B. Yang, H. Kuang, B. Yang, Y. Fu, Y. Cheng, H. Li, Enhanced and sustainable pretreatment for bioconversion and extraction of resveratrol from peanut skin using ultrasound-assisted surfactant aqueous system with microbial consortia immobilized on cellulose, 3 Biotech 10 (2020), https://doi.org/10.1007/s13205-020-02287-1.

[20] International Organization for Standardization, Food and feed products-General guidelines for the determination of nitrogen by the Kjeldahl method, (2009). ⟨www.iso.org⟩.

[21] J.B. Misra, Variation in nitrogen-to-protein conversion factor for peanut, Peanut Sci. 28 (2001) 48–51, https://doi.org/10.3146/i0095-3679-28-2-2.

[22] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956).

[23] A.A. Vilas-Boas, D.A. Campos, C. Nunes, S. Ribeiro, J. Nunes, A. Oliveira, M. Pintado, Polyphenol extraction by different techniques for valorisation of non-compliant portuguese sweet cherries towards a novel antioxidant extract, Sustainability (Switzerland) 12 (2020), https://doi.org/10.3390/su12145556.

[24] T.J. Herald, P. Gadgil, M. Tilley, High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour, J. Sci. Food Agric. 92 (2012) 2326–2331, https://doi.org/10.1002/jsfa.5633.

[25] A. Melo, T.B. Afonso, M.C. Pedrosa, M. Carvalho, C. Rodrigues, M.I. Dias, T. Ribeiro, M. Machado, F. Tavaria, M. Carocho, J.P. Simas, P. Teixeira, L. Barros, M. Pintado, Phenolic profile and antioxidant and biological activities of plant extracts rich in hydrolyzable tannins, ACS Agric. Sci. Technol. 4 (2024) 988–1001, https://doi.org/10.1021/acsagscitech.4c00271.

[26] T.B. Afonso, S. Marçal, P. Vale, A.S. Sousa, J. Nunes, M. Pintado, Exploring the bioactive potential of mushroom aqueous extracts: antimicrobial, antioxidant, and prebiotic properties, Appl. Sci. (Switzerland) 15 (2025), https://doi.org/10.3390/app15031551.

[27] E. Costa, S. Silva, F. Tavaria, M. Pintado, Antimicrobial and antibiofilm activity of chitosan on the oral pathogen Candida albicans, Pathogens 3 (2014) 908–919, https://doi.org/10.3390/pathogens3040908.

[28] E.M. Costa, S. Silva, M. Veiga, P. Baptista, F.K. Tavaria, M.E. Pintado, Textile dyes loaded chitosan nanoparticles: characterization, biocompatibility and staining capacity, Carbohydr. Polym. 251 (2021), https://doi.org/10.1016/j.carbpol.2020.117120.

[29] M. Machado, E.M. Costa, S. Silva, A.M. Gomes, M. Pintado, Exploring the therapeutic potential of avocado oil: Insights into obesity metabolism and immune regulation through in vitro models, Biocatal. Agric. Biotechnol. 63 (2025), https://doi.org/10.1016/j.bcab.2024.103464.

[30] G.C. Braga, P.S. Melo, K.B. Bergamaschi, A.P. Tiveron, A.P. Massarioli, S.M. de Alencar, Rendimento de extração, atividade antioxidante e compostos fenólicos dos subprodutos agro industriais de uva, manga e amendoim, Cienc. Rural. 46 (2016) 1498–1504, https://doi.org/10.1590/0103-8478cr20150531.

[31] A. Mostafa, A. Nassar, N. El-Badry, M. Ammar, The physicochemical and functional properties of red grape and peanut skin powders, Arch. Agric. Sci. J. 0 (2022) 88–103, https://doi.org/10.21608/aasj.2022.236578.

[32] A.M. Shraim, T.A. Ahmed, M.M. Rahman, Y.M. Hijji, Determination of total flavonoid content by aluminum chloride assay: a critical evaluation, LWT 150 (2021), https://doi.org/10.1016/j.lwt.2021.111932.
[33] J. Wang, X. Yuan, Z. Jin, Y. Tian, H. Song, Free radical and reactive oxygen species scavenging activities of peanut skins extract, Food Chem. 104 (2007) 242–250, https://doi.org/10.1016/j.foodchem.2006.11.035.
[34] J. Yu, M. Ahmedna, I. Goktepe, Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics, Food Chem. 90 (2005) 199–206, https://doi.org/10.1016/j.foodchem.2004.03.048.
[35] A. Mostafa, A. Nassar, N. El-Badry, M. Ammar, The physicochemical and functional properties of red grape and peanut skin powders, Arch. Agric. Sci. J. 0 (2022) 88–103, https://doi.org/10.21608/aasj.2022.236578.
[36] L. Zhao, X. Zhu, Y. Yu, L. He, Y. Li, L. Zhang, R. Liu, Comprehensive analysis of the anti-glycation effect of peanut skin extract, Food Chem. 362 (2021), https://doi.org/10.1016/j.foodchem.2021.130169.
[37] M. Monagas, I. Garrido, R. Lebrón-Aguilar, M.Carmen Gómez-Cordovés, A. Rybarczyk, R. Amarowicz, B. Bartolomé, Comparative flavan-3-ol profile and antioxidant capacity of roasted peanut, hazelnut, and almond skins, J. Agric. Food Chem. 57 (2009) 10590–10599, https://doi.org/10.1021/jf901391a.
[38] L.B. Cunha, E.D. Lepore, C.C.B. Medeiros, R. Sorrechia, R.C.L.R. Pietro, M. A. Corrêa, Can gentisic acid serve as a high-performance antioxidant with lower toxicity for a promising new topical application? 14 (2024), https://doi.org/10.3390/life14081022.
[39] S. Youngchim, J.D. Nosanchuk, S. Chongkae, N. Vanittanokom, Ketoconazole inhibits Malassezia furfur morphogenesis in vitro under filamentation optimized conditions, Arch. Dermatol. Res. 309 (2017) 47–53, https://doi.org/10.1007/s00403-016-1701-4.
[40] X. Yang, B. Xiong, Z. Yuan, H. Liao, X. Liu, Y. Wu, S. Zhang, Q. Xiang, Polygalaxanthone III, an Active Ingredient in Polygala japonica Houtt., Repaired Malassezia-Stimulated Skin Injury via STAT3 Phosphorylated Activation, Molecules 27 (2022), https://doi.org/10.3390/molecules27217520.
[41] T. Tamura, M. Ozawa, N. Tanaka, S. Arai, K. Mura, Bacillus cereus response to a proanthocyanidin trimer, a transcriptional and functional analysis, Curr. Microbiol. 73 (2016) 115–123, https://doi.org/10.1007/s00284-016-1032-x.
[42] P.J. Sarnoski, R.R. Boyer, S.F. O'Keefe, Application of proanthocyanidins from peanut skins as a natural yeast inhibitory agent, J. Food Sci. 77 (2012), https://doi.org/10.1111/j.1750-3841.2012.02652.x.
[43] R.P. Pizzolitto, J.S. Dambolena, M.P. Zunino, M. Larrauri, N.R. Grosso, V. Nepote, A.M. Dalcero, J.A. Zygadlo, Activity of natural compounds from peanut skins on Fusarium verticillioides growth and fumonisin B1 production, Ind. Crops Prod. 47 (2013) 286–290, https://doi.org/10.1016/j.indcrop.2013.03.020.
[44] I.A. Eydelnant, N. Tufenkji, Cranberry derived proanthocyanidins reduce bacterial adhesion to selected biomaterials, Langmuir 24 (2008) 10273–10281, https://doi.org/10.1021/la801525d.
[45] M. Daglia, M. Stauder, A. Papetti, C. Signoretto, G. Giusto, P. Canepari, C. Pruzzo, G. Gazzani, Isolation of red wine components with anti-adhesion and anti-biofilm activity against Streptococcus mutans, Food Chem. 119 (2010) 1182–1188, https://doi.org/10.1016/j.foodchem.2009.08.037.
[46] R.K. Ulrey, S.M. Barksdale, W. Zhou, M.L. Van Hoek, Cranberry proanthocyanidins have anti-biofilm properties against pseudomonas aeruginosa, BMC Complement. Altern. Med. 14 (2014), https://doi.org/10.1186/1472-6882-14-499.
[47] P.A. Pinzón-Arango, K. Holguin, T.A. Camesano, Impact of cranberry juice and proanthocyanidins on the ability of Escherichia coli to form biofilms, Food Sci. Biotechnol. 20 (2011) 1315–1321, https://doi.org/10.1007/s10068-011-0181-8.
[48] D. Nicolosi, G. Tempera, C. Genovese, P.M. Furneri, Anti-adhesion activity of A2-type proanthocyanidins (a cranberry major component) on uropathogenic E. coli and P. mirabilis strains, Antibiotics 3 (2014) 143–154, https://doi.org/10.3390/antibiotics3020143.
[49] A.B. Howell, J.D. Reed, C.G. Krueger, R. Winterbottom, D.G. Cunningham, M. Leahy, A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity, Phytochemistry 66 (2005) 2281–2291, https://doi.org/10.1016/j.phytochem.2005.05.022.
[50] A. Prokop, A. Magiera, M.A. Olszewska, Proanthocyanidins as therapeutic agents in inflammation-related skin disorders, Int. J. Mol. Sci. 26 (2025), https://doi.org/10.3390/ijms262010116.
[51] Y.E. Rossi, L.P. Bohl, N.L. Vanden Braber, M.B. Ballatore, F.M. Escobar, R. Bodoira, D.M. Maestri, C. Porporatto, L.R. Cavaglieri, M.A. Montenegro, Polyphenols of peanut (Arachis hypogaea L.) skin as bioprotectors of normal cells. Studies of cytotoxicity, cytoprotection and interaction with ROS, J. Funct. Foods 67 (2020), https://doi.org/10.1016/j.jff.2020.103862.
[52] J.M. Jaffri, Reactive oxygen species and antioxidant system in selected skin disorders, Malays. J. Med. Sci. 30 (2023) 7–20, https://doi.org/10.21315/mjms2023.30.1.2.
[53] Y. Dong, Z. Wang, ROS-scavenging materials for skin wound healing: advancements and applications, Front. Bioeng. Biotechnol. 11 (2023), https://doi.org/10.3389/fbioe.2023.1304835.