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Phytochemical profiling and investigation of antioxidant, anti-proliferative, and antibacterial properties in spontaneously grown Sicilian sumac (*Rhus coriaria* L.) fruits

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ABSTRACT

This study aimed to assess the phytochemical profile and associated functional properties of sumac (*Rhus coriaria* L.) fruits harvested from wild plants in Sicily.

Chemical characterization unveiled exceptionally high levels of polyphenols (10.99 g GAE/100 g DW), including substantial quantities of proanthocyanidins (61.577 mg PACE/100 g DW), and identified 82 phytochemicals belonging to various flavonoid classes. The hydroalcoholic extract from Sicilian sumac exhibited remarkable redox-active properties, providing antioxidant protection in a cell-based model of lipid peroxidation (CAA50: 1.116 μg/mL). Additionally, it displayed significant antiproliferative activity against four human tumor epithelial cell lines with GI50 values ranging from 31.08 to 149.74 μg/mL and robust antibacterial activity against major foodborne pathogens (MIC: 12.5–25.0 mg/mL).

Our findings highlight Sicilian sumac fruit as a rich source of phytochemicals that positively contribute to the cellular redox state even when consumed in small quantities. Additionally, its diverse bioactivities indicate potential applications across food and non-food sectors.

1. Introduction

In the vast field of nutritional science, the importance of dietary phytochemicals in promoting well-being and preventing chronic diseases is widely recognized. Numerous experimental studies have revealed a diverse range of biological activities associated with these plant-derived compounds, demonstrating their potential benefits for human health (Salehi et al., 2020). Furthermore, the utilization of phytochemicals extends beyond health promotion to enhance the safety and functional properties of food products. This has led to the incorporation of various plant-derived products, including those derived from native Mediterranean plants, in food processing applications. Such applications aim to increase the antioxidant content of food items and alleviate the adverse effects of microbial contamination (Grigoriadou et al., 2020).

Within the Anacardiaceae family, the *Rhus* genus, commonly known as Sumac, stands out as one of the largest genera with over 250 species distributed across temperate and tropical regions globally. *Rhus* species have a rich history in traditional herbal medicine across diverse cultures, used to treat various ailments. Despite numerous studies exploring the phytochemical profile and biological activity of different parts of various *Rhus* species, there is still much to discover about the functional value of *Rhus coriaria*, the typical sumac of the Mediterranean area (Batiha et al., 2022). Several studies have emphasized the biological activity of various parts of *Rhus coriaria*, especially its fruit, demonstrating antimicrobial, antioxidant, and anti-inflammatory properties, as well as positive effects on blood glucose concentration and serum lipid profiles (Alsamri et al., 2021). Despite, existing research emphasizes significant biodiversity in the phytochemical profile and associated biological activity within *R. coriaria*, depending on geographical origin (Mazzara et al., 2023), for

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our knowledge few studies have effectively correlated biodiversity with phytochemical profiling and observed biological activities.

In Sicily, the once-cultivated sumac is known as “Sicilian sumac,” and its dried and ground leaves were historically used as a colorant for tanning leather. Presently, it grows spontaneously in uncultivated lands and is exclusively utilized as a culinary herb and spice to flavor fish, meat, and salads (Calabrò et al., 2023). A recent study by Mazzara et al. (2023) has highlighted significant diversity in hydroalcoholic extracts from sumac fruits obtained from various Sicilian populations (Mazzara et al., 2023). Although the study by Mazzara et al. (2023) represents one of the most comprehensive works on Sicilian sumac available in the scientific literature to date, its sole focus is on the changes in the major bioactive compounds in five sumac genotypes through spectroscopic analyses, alongside potential alterations in antioxidant activity assessed through a single spectroscopic assay. Consequently, more in-depth investigations aiming to provide a broader understanding of reducing activity, both in other solution systems and in cellular models, are warranted. Furthermore, information regarding biological activities other than antioxidant activity (i.e. antimicrobial antiproliferative) is lacking.

This study is part of a broader initiative aimed at rediscovering the cultivation of Mediterranean endemic plants within the rural Sicilian landscape, with a specific focus on their potential applications in producing innovative and health-oriented foods. In our research, we aimed to fill this gap concerning Sicilian sumac by conducting a thorough analysis of its bioactive compounds using advanced chromatographic techniques (HPLC-DAD-MS/MS) alongside spectroscopic methods (UV/Vis). Our approach went beyond mere compound identification; we also provided quantitative insights into each identified species. Moreover, we shed light on its potential biological activities through assays designed to evaluate radical scavenging and reducing activities, complemented by an antioxidant assessment system utilizing a biologically relevant cellular model (CAA assay). Beyond the biochemical approach, our study also endeavors to elevate the status of spontaneously grown Sicilian sumac by investigating its associated antimicrobial and antiproliferative properties, which hold promise for applications in human health. To our knowledge, no prior literature comprehensively addresses the potential biological activities of sumac species. Hence, this work aims to underscore the value of a relatively unknown product not only beyond Sicilian borders but also within the region itself.

2. Materials and methods

2.1. Plant materials and sumac fruits powder production

Sumac fruits were harvested at their optimal ripeness from two distinct locations within the Regional Park of the Madonie mountains near Palermo, Sicily. These locations included the area of Gratteri, and Collesano, both on limestone rocks in uncultivated lands near Mediterranean maquis. It is noteworthy that all samples were expertly collected and identified by Professor Rosario Schicchi.

The plant specimens were deposited at the Herbarium Mediterraneum Panorminatum of the Botanical Garden of the University of Palermo (Orto Botanico, Palermo, Italy) with the voucher numbers 109724–109725.

The fruits were manually collected in September 2022 using disposable gloves and dissecting scissors. After harvesting, the fruits were placed into sterile plastic bags (Corning Gosselin sas, Borre, France) and transferred to the laboratory of Agricultural Microbiology of the Department of Agricultural, Food and Forest Science (SAAF) – University of Palermo (Italy). They were then dried in a drying chamber (Binder GmbH, Tuttlingen, Germany) at 54 °C for 48 h and milled using a Fritsch Pulverisette 14 mill (Fritsch GmbH, Idar-Oberstein, Germany) to obtain power sumac fruits (PSF) with a size of 250 μm.

2.2. Hygienic and safety aspects of PSF

The microbiological evaluation of PSF was performed to detect the presence of the main pathogenic and spoilage microorganisms as reported by Viola et al. (2023). Ten grams of powder was homogenized in 90 mL of Ringer’s solution (Sigma-Aldrich, Milan, Italy), serially diluted in the same diluent, and plated on: Plate Count Agar (PCA) (Biotec, Grosseto, Italy) incubated for 72 h at 30 °C for the enumeration of total mesophilic microorganisms (TMM); *Pseudomonas* Agar Base (PAB) (Oxoid, Hampshire, UK) incubated for 48 h at 25 °C for *Pseudomonas* spp.; Violet Red Bile Glucose Agar (VRBGA) (Condalab, Madrid, Spain) incubated for 24 h at 37 °C for members of the Enterobacteriaceae family; Violet Red Bile Agar (VRBA) (Microbiol Diagnostici, Uta, Italy) incubated for 24 h at 37 °C for total coliforms; Baird Parker (BP) agar incubated for 48 h at 37 °C for coagulase-positive staphylococci (CPS); Agar *Listeria* to Ottaviani & Agosti (ALOA) (Biolife Italiana, Monza, Italy) incubated for 24 h at 37 °C for *Listeria monocytogenes*; Hektoen Enteric Agar (HEA) (Microbiol Diagnostici) incubated for 24 h at 37 °C for *Escherichia coli* and *Salmonella* spp.; Dichloran Rose-Bengal Chloramphenicol (DRBC) agar (Microbiol Diagnostici) incubated for 48 h at 30 °C for yeasts; Potato Dextrose Agar (PDA) (Microbiol Diagnostici) incubated for 7d at 25 °C for molds. Cell suspensions on both Violet Red Bile Glucose Agar (VRBGA) and Violet Red Bile Agar (VRBA) were pour plated on double-layered agar, while on all other agar media were spread plated. PSF was also analyzed for spore-forming aerobic bacteria heating cell suspension for 15 min at 85 °C, spread plated onto Nutrient Agar (Oxoid) and incubated at 32 °C for 48 h. All Petri dishes were incubated aerobically. Analyses were performed in duplicate.

2.3. Preparation of PSF extract

To prepare the extract, three separate portions of *Rhus coriaria* fruits were weighed and subjected to extraction using a mixture comprising 70:30 (v/v) ethanol:water at a extraction ratio of 1:10 (w/v). The samples were vortexed for 5 min, sonicated at 40 KHz (Giorgio-Bormac, Modena, Italy) at room temperature for 20 min, and stirred on a plate shaker for 24 h at 4 °C in the dark. Following this, the samples underwent centrifugation at 2000 rpm for 10 min at 4 °C. The extraction protocol was repeated four time, and the supernatants from both cycles were collected and filtered through Millex HV 0.45 μm filters (Millipore, Billerica, MA). Meanwhile, the residue underwent an additional extraction with the same solvent, and the resulting supernatant was stored separately as an exhausted extract. The obtained extract was preserved at −20 °C until further chemical and biological analyses.

2.4. Phytochemical characterization

2.4.1. Total polyphenol content

Phenolic content of the extract from PSF was determined via Folin-Ciocalteu method, with some minor changes as previously reported (Rumpf et al., 2023). Gallic Acid (GA) was used as reference standard and the results were expressed as mg GA Equivalents (GAE) per 100 g of DW. The experiments were repeated three times.

2.4.2. Total proanthocyanidin content

The Total Proanthocyanidins Content (TPAC) in the extract was evaluated using a modified protocol of the BL-DMAC assay as previously reported (Mannino et al., 2021a). TPAC value was expressed as mg Proanthocyanidin A-type (PAC-A) equivalent per 100 g of DW. The experiments were repeated three times.

2.4.3. Total anthocyanin content

The total anthocyanin content (TAC) in the extract was measured through the pH differential method (Feliciano et al., 2012), excluding potential interference of other colored pigments. Briefly, an appropriate dilution of the extract was added to 0.025 M KCl (pH 1.0) or 0.4 M

sodium acetate (pH 4.5) buffer. The absorbance of both reaction mixture was read at 510 nm and 700 nm against the respective blanks. TAC value was determined using the following equation (Equation (1)):

$$TAC\ (mg/ml) = \frac{(Abs_{510} - Abs_{700})_{pH1} - (Abs_{510} - Abs_{700})_{pH4.5}\ x\ MW\ x\ 1000}{\varepsilon\ x\ l} \quad (1)$$

Where: MW is the molecular weight of cyanidin-3-glucoside (449.2 g mol−1); ε is the molar extinction coefficient (26,900 mM^{-1} mol^{-1}) of cyanidin-3-glucoside; l is the path length (1 cm). Data were expressed as mg of cyanidin -3-glucoside equivalent (CE) per 100 g of DW. The experiments were repeated three times.

2.4.4. Total carotenoid content

Three separate 3-g aliquots of PSF were extracted for 20 min with 50 mL hexane. After a cleanstep by centrifugation at 2000 rpm for 10 min at 4 °C, the extraction protocol was repeated a second time. The supernatants from both cycles were collected and was used for analysis following appropriate dilution with hexane. The determination of the total carotenoid content (TC) involved measuring the absorbance at 460 nm and 480 nm on a portion of the hexane extract (Albuquerque et al., 2016). TC was calculated using β-carotene as reference standard and expressed as mg β-carotene per 100 g of DW. All measurements were performed in triplicate for accuracy.

2.4.5. Phytochemical composition

The HPLC system used an Agilent Technologies model 1200 high-performance liquid chromatography (HPLC) instrument interfaced with a diode array detector (DAD) and a tandem mass spectrometry (MS/MS) system equipped with an Agilent 6330 series LC-MS ion trap. The chromatographic separation utilized a reverse-phase C18 Luna column (3.00 μm particle size, 150 mm × 3.0 mm internal diameter, Phenomenex) operated at a constant flow rate of 0.2 mL min-1 and maintained at a temperature of 25 °C within an Agilent 1100 HPLC G1316A Column Compartment. For flavonoid identification and quantification, tandem mass spectrometry analyses were conducted in negative ionization mode, while for anthocyanins, positive ionization mode was employed. These conditions were optimized for the accurate determination of these compounds, as previously reported (Vigliante et al., 2019). For flavonoids, a binary solvent system comprised MilliQ water acidified with 0.1% (v/v) formic acid (solvent A) and acetonitrile acidified with 0.1% (v/v) formic acid (Solvent B). The initial solvent composition consisted of 90% (v/v) A and 10% (v/v) B for the first 5 min of the chromatographic run. Subsequently, the proportion of solvent B was gradually increased to 70% (v/v) over the next 50 min. Following each chromatographic run, the initial solvent composition was restored for an additional 10 min before the next injection, ensuring consistent chromatographic performance. The sample injection volume for each analysis was 10 μL. UV/Vis spectra were recorded across the range of 220–650 nm to monitor the progress of the separation. The chromatographic profiles were monitored at 220, 260, 280, and 360 nm. For anthocyanin analysis, a slightly different binary solvent system was employed, involving MilliQ water containing 10% (v/v) formic acid (Solvent C) and 50% (v/v) methanol acidified with 10% (v/v) formic acid (Solvent D). The elution method employed a multistep linear solvent gradient: 0–5 min, 10% (v/v) B; 5–15 min, linear increase to 45% (v/v) B; 15–20 min, linear increase to 60% (v/v) B; 20–25 min, linear decrease to 10% (v/v) B. The total analysis time was 25 min, including a 5-min equilibration period, minimizing potential for peak distortion. The sample injection volume was 20 μL of solution. UV-VIS spectra were registered at 520 nm, a wavelength that exhibits strong absorption by anthocyanins, enabling accurate quantification of these compounds.

2.5. Redox active properties

The redox active properties of the extract from PSF were estimated using three in solution assay. In particular, the radical-scavenging activity was measured via ABTS and DPPH assays, while the metal-reducing antioxidant capacity was evaluated via ferric reducing antioxidant power (FRAP) assay (Rumpf et al., 2023). For each in solution assay the results were obtained from an average of three separate experiments. Trolox served as the standard reference, and the reducing activity assessed by each assay was expressed as mmol of Trolox Equivalent (TE) per 100 g of dry weight (DW).

2.5.1. ABTS assay

The green-stable cationic radical $ABTS^{\bullet+}$ was freshly prepared by incubating ABTS salt with $K_2S_2O_8$ at room temperature overnight. Subsequently, varying concentrations of the extract were added to the previously diluted $ABTS^{\bullet+}$ radical in ethanol. After 3 min of incubation at RT, the reduction of the $ABTS^{\bullet+}$ radical was tracked spectrophotometrically at 734 nm.

2.5.2. DPPH assay

DPPH radical solution was combined with 10 μL of appropriately diluted extract. After a 20-min incubation period, the absorbance was measured using a microplate reader at 517 nm using ethanol as a blank.

For both the assays, the discoloration percentage (D) of the radical solution was determined using Equation (2):

$$D = \frac{A_{CTR} - A_{TEST}}{A_{CTR}}\ x\ 100 \quad (2)$$

Where: D is the percentage of color reduction of the radical solution; A_{CTR} is the absorbance of the ABTS•+ (734 nm) or DPPH (517 nm) radical solution before the addition of the extract; A_{TEST} is the absorbance of the $ABTS^{\bullet+}$ (734 nm) or DPPH (517 nm) radical solution after the addition of the extract at the end of incubation time.

2.5.3. FRAP assay

For Ferric Reducing Antioxidant Power (FRAP), a mixture containing 300 mM sodium acetate with 20 mM $FeCl_3$ and 10 mM TPTZ in an 8:1:1 (v/v/v) ratio was prepared. Subsequently, this reaction buffer was incubated at 37 °C for 1 h with the appropriately diluted extract. Following the incubation period, the absorbance of each well was measured at 595 nm against a blank. using a microplate reader.

2.6. Cellular antioxidant activity

The cellular antioxidant activity (CAA) assay was performed as previously described (Wolfe & Liu, 2007). HepG2 cells were seeded in complete culture medium at high density. After 24 h of incubation in cell culture conditions, the cells were exposed for 2 h to both DCFH-DA (Sigma Aldrich, St. Louis, Missouri, USA) and appropriate dilution of the extract from PSF. Ethanol concentration never exceeded 0.25% (v/v) and cell medium containing 0.25% (v/v) ethanol was used as control. Then, cells were washed with PBS and oxidative stress was induced exposing the cells to ABAP (Sigma Aldrich, St. Louis, Missouri, USA) in HBSS (Sigma Aldrich, St. Louis, Missouri, USA). HBSS alone was added to blank wells. The fluorescence was evaluated every 5 min for 1 h by using a plate-reader at 37 °C. The CAA value was calculated using Equation (3):

$$CAA = 100 - \left[100 \times \left(\frac{\int SA}{\int CA}\right)\right] \quad (3)$$

Where: $\int$ SA is the integrated area of the wells incubated with sumac extract; $\int$ CA is the integrated area of the control wells.

The antioxidant activity of the extract from PSF was expressed as CAA_{50} that is the extract concentration necessary for 50% of DCF

formation inhibition. CAA_{50} was calculated from a concentration-response (CAA) curve using linear regression analysis, and it was expressed as mg of DW per mL of cell medium. The result is the mean value of three separate experiments.

2.7. Antiproliferative activity

The human epithelial cell lines HeLa (human cervical cancer), Caco-2 (human colon cancer), HepG2 (human hepatocarcinoma), and MCF-7 cells were obtained from American Type Culture Collection (Rockville, MD, USA). Caco-2 cells and MCF-7 cells were grown in DMEM GlutaMax while HeLa and HepG2 cells in RPMI. Both culture media were supplemented with 10% (w/v) FBS, 2 mM L-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin and maintained in a humidified atmosphere with 5% (v/v) CO_2 at 37 °C. Cells were mostly cultured in 75 cm^2 culture flasks and were trypsinized using trypsin-EDTA before the confluence was reached.

MTT assay was performed on exponentially growing cells as previously reported (Gentile et al., 2016). Cells were seeded into standard 96-well plates at a density depending on the doubling times of each cell line. After 24 h of incubation, the extract from PSF at appropriate concentrations (100-10 μg DW/mL cell culture medium) was added. Ethanol concentration never exceeded 0.25% (v/v) and control cells were incubated with culture medium containing 0.25% ethanol (v/v). After 48 h of incubation, MTT reagent was added and discarded after a 3 h incubation. The produced blue formazan was dissolved by dimethyl sulfoxide (DMSO) and the absorbance at 570 nm was recorded using a microplate reader. The percentage of growth (PG) of cells exposed to the extract with respect to control cells (untreated cells) was obtained using the following Equation (4):

$$PG = 100\,x\,\frac{(ODS - OD_{tzero})}{(ODC - OD_{tzero})} \tag{4}$$

Where: ODS is the average of optical density after cell exposure to the sumac extract for a chosen period of time; ODtzero is the average of optical density before the extract addition; ODC is the average of optical density after the chosen period of time with no exposure of cells to treatment.

The concentration needed to induce 50% growth inhibition (GI_{50}) for each cell line was determined from concentration-response (PG) curves using linear regression analysis, and it was expressed as mg of DW per mL of cell medium. The results, expressed as μg/mL of cell medium, is the mean value of three separate experiments.

2.8. Evaluation of antibacterial activity

The antibacterial properties of PSF extract were tested against the main food-borne pathogenic bacteria. Specifically, the strains *Bacillus cereus* ICE170, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19114, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Enteritidis ATCC13076; Salmonella Typhimurium 50432 and Staphylococcus aureus ATCC33862 were used as indicator (sensitive) bacteria. Non-ATCC (American Type Culture Collection) belonged to the bacterial culture collection of the SAAF Department. All indicator strains were sub-cultured in Brain Heart Infusion (BHI) broth (Oxoid) in which they reached a cell density of approximately 10^9 CFU/mL after incubation at 37 °C for 24 h. The antibacterial activity was evaluated by the well diffusion assay (WDA) as reported by Barbaccia et al. (2022). Briefly, BHI soft agar containing the indicator strains at a cell density of 10^7 CFU/mL was overlaid on an agar-water support. Circular wells were made using a sterile 6-mm cork borer and then filled with PSF extract. Sterile filter paper discs (Whatman no. 1) of the same diameter were soaked with streptomycin (10% w/v) as positive control and mixture 70:30 (v/v) ethanol:water as negative control. Plates were incubated at 37 °C for 24 h, and the antibacterial activity was scored positive only in the presence of an inhibition zone around the wells. The WDA test was performed in duplicate.

2.9. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the PSF extract against the sensitive bacteria was quantified in 96-well microplates following the pervious described approach (Barbaccia et al., 2022). The ability of pathogenic bacteria to grow in diluted PSF extract at concentrations between 100 and 6.25 mg/mL was measured at 0 and 24 h after inoculation using a P-800 ScanReady Spectrophotometer (Life Real Biotechnology Co., Ltd, Hangzhou, China). The MIC test was performed in duplicate.

2.10. Statistical analyses

All results were expressed as mean ± standard deviation (SD). Student t-test or one-way ANOVA followed by Tuckey's post hoc test was performed to assess significance among different experimental conditions. All statistical analyses were carried out using SPSS Statistics 24 (SSPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Safety criteria of PSF

According to the International Commission on Microbiological Specifications for Foods, spices, herbs, and dried vegetable seasonings must not exceed a limit of 10^4 CFU/g of total bacteria at 30 °C (Commission, 2005). Hence, the PSF produced in this study underwent microbiological evaluation to ascertain its hygienic suitability as a natural food additive. Such assessment is imperative prior to the utilization of dried-plant products in food applications, given their potential role as carriers for foodborne pathogens (Sagoo et al., 2009). The microbiological analysis conducted using a culture-dependent approach did not reveal the presence of spoilage or pathogenic populations (data not shown), indicating the hygienic suitability of PSF for use as an additive in processed foods. Notably, the presence of spoilage microorganisms such as pseudomonads, yeasts, and molds, as well as pathogenic bacteria including aerobic spore-forming bacteria, *E. coli*, coagulase-positive staphylococci, *L. monocytogenes*, and Salmonella spp., in a natural product intended for use as an additive, can adversely impact the quality and safety of the foods into which it is incorporated (Dlugaszewska et al., 2019). Nevertheless, the absence of these microorganisms in PSF is undoubtedly attributable to the high-quality standards implemented throughout the entire process, from harvesting to processing (Cicero et al., 2022).

3.2. UV/Vis phytochemical characterization

The phytochemical profile of PSF was assessed through a combination of colorimetric assays and HPLC techniques. Initially, the Folin reagent was employed to quantify the total polyphenolic content (TPC) of the hydroalcoholic extract. Two additional colorimetric assays, DMAC and the pH jump method, were utilized to estimate the total content of two major polyphenolic classes in the same extract: proanthocyanidins (TPAC) and anthocyanins (TAC), respectively. The total carotenoid content, expressed as μg-carotene equivalents, was estimated from the maximum absorbance at 435 nm of PSF hexane extract (Table 1).

The estimated TPC value, surpassing those reported for all fruits documented in the Phenol-Explorer Database, including black raspberry (980 mg/100 g) and blackcurrant (821 mg/100 g), indicating that Sicilian sumac is an exceptional source of polyphenolic compounds (Díaz-García et al., 2013). The intense coloration of sumac fruits can be attributed to a significant anthocyanin content, as highlighted by the

Table 1
Bioactive compounds and antioxidant properties of Sumac extract. Values are expressed as mean ± SD of three experiments carried out in triplicate.

TPC	10.99 ± 0.06	*g GAE* per *100 g of DW*
TPAC	61.58 ± 5.73	*mg PACE* per *100 g of DW*
TAC	79.52 ± 4.12	*mg CE* per *100 g of DW*
TCC	1.74 ± 1.10	*mg BCE* per *100 g of DW*
DPPH	124.49 ± 0.98	*mmol TE* per *100g of FW*
ABTS	106.91 ± 6.11	*mmol TE* per *100g of DW*
FRAP	91.81 ± 15.30	*mmol TE* per *100g of DW*
CAA_{50}	1.12 ± 0.09	*ug of DW* per *mL cell medium*

Abbreviations: Total polyphenol content (TPC), total anthocyanin content (TAC), total proanthocyanidin (TPAC), total carotenoid content (TCC), ferric reducing antioxidant power (FRAP), cellular antioxidant activity (CAA), gallic acid equivalents (GAE), proanthocyanidin A type equivalents (PACE), cyanidin glucoside equivalents (CE), β-caroten equivalents (BCE).

high TAC value estimated in the hydroalcoholic extract of PSF, comparable to that determined for red fruits such as jostaberry (74 mg/100 g) and lingonberry (48.88 mg/100 g) (Mannino, Gentile, et al., 2021). The obtained results align with findings by others, who explored the total polyphenol and anthocyanin content of hydroalcoholic extracts from sumac fruits from other geographic origins. Kosar et al. (2007) assessed the total polyphenolic content and antioxidant activity of hydroalcoholic extracts from defatted Turkish sumac fruits, estimating a TPC value that is half of the TPC value determined for Sicilian sumac fruits in our study (Kosar et al., 2007). Conversely, comparable is TAC value between Sicilian and Turkish sumac. In a very recent study, Mazzara et al. (2023) evaluated the functional value of four samples of Sicilian sumac from five different regions, including regions distinct from the one our sample originates from (Mazzara et al., 2023). They demonstrated significant variability both in terms of bioactive compound content and antioxidant activity. Moreover, the TPC value estimated for our sample is more than double that determined for the sumac samples in Mazzara et al. (2023) study. In contrast, our sample exhibits an anthocyanin content, though comparable or higher than some of the samples analyzed by Mazzara et al. (2023), distinctly lower than that determined for other samples in the same study.

Proanthocyanidins are widely distributed polyphenolic polymers in nature and the most abundant flavonoids consumed in the diet, also known as condensed tannins to distinguish them from less common ellagitannins or hydrolysable tannins (Mannino et al., 2021a). The biological activity of these polyphenols has been linked not only to their redox-active properties but also to their ability to interact with various biological targets, influencing their function. These properties justify the documented antimicrobial, antiviral, anti-inflammatory, and hypoglycemic activities of extracts rich in proanthocyanidins (Mannino et al., 2021a). Despite the highlighted high content of hydrolysable tannins in sumac fruits, the presence of condensed tannins had never been demonstrated (Abu-Reidah et al., 2015).

Phytochemical analysis, both colorimetric methods and HPLC, allow us to find presence of proanthocyanidins in the hydroalcoholic extract of Sicilian sumac. The TPAC value, estimated by BL-DMAC assay, indicates a content of proanthocyanidins higher to the TPAC content in apples (21.2 mg/100 g), plums (62.50 mg/100 g), and strawberries (65.85 mg/100 g) (Mannino et al., 2021a). On the other hand, considering the high stability of these polymers under gastrointestinal digestion conditions, it is predictable that the consumption of small quantities of sumac could lead to significant concentrations in the intestinal lumen. Through local actions on intestinal epithelial cells, this could potentially enhance the physiology of the gastrointestinal tract (Mannino et al., 2021a).

Finally, we also assessed the presence of carotenoids in our sample of Sicilian sumac. Carotenoids are a group of pigments responsible for the red, orange, and yellow colors in many fruits and vegetables. They also possess antioxidant properties and contribute to human health. The total carotenoid content in the lipophilic fraction of our sample, expressed as beta-carotene equivalent (BCE), is 1.5 mg/100 g. This value is relatively low, especially when compared to the content in other more widely consumed spices (Gaglio et al., 2019).

3.3. HPLC-DAD-ESI-MS/MS characterization

This research delves into the ethanolic extract from Sicilian Sumac (*Rhus* spp.) utilizing High-Performance Liquid Chromatography coupled with Diode Array Detector and Tandem Mass Spectrometry (HPLC-DAD-MS/MS). Identification and quantification (expressed as mg per 100 g of FW) are reported in Fig. 1. The analysis successfully identified 82 bioactive compounds, spanning various flavonoid classes (Fig. 1). Notably, six of these compounds were anthocyanins, three of which exhibited simple –OH groups on the B-ring of the flavonoid scaffold (Idaein, Cyanidin, and Chrysanthemin), while the remaining trio featured a methyl substitution on one of these groups, namely petunidin and its glycosidated derivatives. While anthocyanins constitute only 7% (w/w) of the total identified polyphenols in the extract, this seemingly modest proportion is sufficient to bestow upon it the renowned red and vibrant pigmentation of Sumac. Moreover, thanks to the antioxidant properties of anthocyanins, their presence in Sumac extracts not only enhances the visual appeal of the plant material but also hints at potential health benefits linked to their antioxidant activity (Mannino et al., 2021b).

Concerning flavan-3-ols, they represented more than 15% (w/w) of the total identified flavonoids in the Sumac extract. Qualitative analysis identified five glycosidated flavan-3-ols, predominantly comprising forms of catechin and epicatechin mono- or di-glucosides, in addition to eight polymeric forms of flavan-3-ols, specifically proanthocyanidins. Proanthocyanidins, a distinctive class of polyphenolic compounds, are noteworthy for their restricted distribution in the plant kingdom. Unlike other flavonoids, proanthocyanidins are notably present only in specific plant families, such as legumes, Ericaceae, and select fruit species (Mannino et al., 2021a). The presence of proanthocyanidins in *Rhus coriaria* is particularly intriguing as proanthocyanidins often play a role in the formation of more complex aggregates, recognized as condensed tannins, which confer significant resistance to environmental factors and herbivore interactions (Mannino et al., 2021a; Rauf et al., 2019).

Despite the *cis*-trans geometry of flavan-3-ols and proanthocyanidins being challenging to discern through chromatography, the coexistence of proanthocyanidins and anthocyanins in *Rhus coriaria* suggests the presence of a stereospecific biosynthetic pathway that generates flavan-3-ols and anthocyanins through the reduction or oxidation of their respective leucoanthocyanins. The high stereospecificity of the enzymes involved in this process results exclusively in flavan-3-ols in the *cis*-conformation. This metabolic specificity enhances the diversity and complexity of *Rhus coriaria*'s phenolic compounds, potentially imparting unique properties to Sumac. These characteristics make it a valuable resource for the food, pharmaceutical, or cosmetic industries, given the diverse range of benefits associated with these compounds, including antioxidant and anti-inflammatory properties (Mannino et al., 2021a).

The predominant class of compounds within the Sumac extract was unquestionably flavonols, both in terms of quantity and quality. Out of the 82 identified compounds, 34 belong to this specific class of flavonoids, constituting approximately 41% (w/w) of the total identified flavonoid quantity. The most prevalent compounds in this category included Afzelin, the glycosidated form of fisetin, myricitroside, quercetin, and isoquercitrin. Unlike other flavonols with well-documented distribution in the plant kingdom, the detection of fisetin within the hydroalcoholic extracts of Sumac and its quantification in discernible amounts are particularly intriguing. Preliminary research suggests that Fisetin may have a positive impact on brain function and cognitive health. Studies on animal models indicate that this flavonoid could positively influence memory and cognitive functions, presenting promising possibilities for its application in neuroprotection and the prevention of neurodegenerative disorders like Alzheimer's (Kumar et al.,

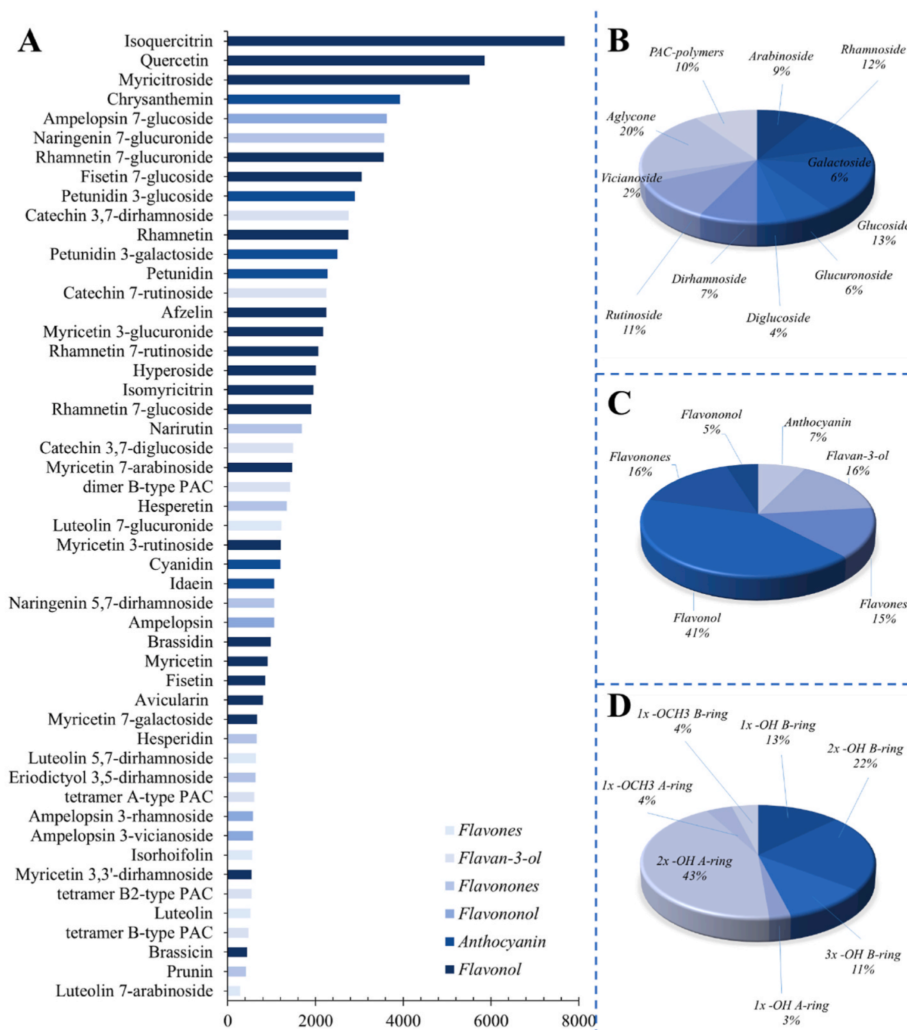

Fig. 1. Chemical composition profile of compounds identified in the hydrophilic extract of Sumac. In Panel A, the top 50 compounds are highlighted, representing diverse classes of flavonoids (flavonols, anthocyanins, flavononols, flavones, flavan-3-ols, and flavones). Values are reported as mg of compound per 100 g of dried weight. The complete list of all identified and quantified compounds can be found in Supplementary Table S1, Chromatograms in Fig. S1, and Raw Data including MS/MS fragmentation spectra in Supporting Data File 1. Panel B quantifies the identified compounds present as aglycones, flavan-ol polymers (PAC), or associated with a sugar moiety. Panel C categorizes the identified compounds into various flavonoid families. Panel D enumerates the hydroxyl (-OH) or methoxyl (-OCH_3) substituents linked to the A or B ring of the flavonoid scaffold.

2023). Consequently, it is crucial to underscore that its presence in plants like Sumac enriches the diversity of bioactive compounds within these native Mediterranean plant species.

Concerning flavones and flavonones, these compounds collectively contribute approximately 30% (w/w) to the total identified polyphenols in the extract. Notably, cosmetin, skolimoside, and apigenin play pivotal roles in this contribution. Cosmetin and skolimoside, also recognized as luteolin 7-*O*-glucoside and luteolin-7-*O*-rutinoside, respectively, exhibit a flavone backbone with a distinctive glycoside moiety positioned at the 7-O site. Abundant in the Rhus genus, these compounds initially found applications in cosmetics due to their antioxidant properties but have garnered increased significance for their demonstrated ability to suppress iNOS and COX-2, unveiling promising anti-inflammatory properties (Bangar et al., 2023; Çetinkaya & Baran, 2023). Apigenin, widely distributed in various plants, boasts a flavone structure with specific hydroxyl group positions, rendering it remarkably versatile. In contrast to the aforementioned flavones, apigenin is a glycoside featuring a singular hydroxyl group on the B ring of the flavonoid scaffold, currently under investigation for its cancer preservation activity. Turning attention to flavonones, the exclusive representatives of this class are the glycosidate forms of eryodictol and naringenin. Despite their relative scarcity, flavonones constitute a noteworthy 16% (w/w) of the identified flavonoids, underscoring their unique contribution to the phytochemical profile.

3.4. Antioxidant properties

Oxidative species, generated during normal aerobic metabolism, play essential roles in various cellular processes, including proliferation, differentiation, and apoptosis. However, an imbalance between endogenous antioxidant defenses and the production of reactive species leads to cellular oxidative damage, significantly contributing to the development and progression of various chronic diseases (Curieses Andrés et al., 2023). The antioxidant activity of phytochemicals makes them effective in enhancing endogenous antioxidant defense systems. This is related not only to the scavenging activity of reactive species but also to the demonstrated ability to modulate the expression and activity of antioxidant enzymes (Sharma et al., 2024).

To assess the ability of the Sicilian Sumac extract to neutralize reactive species by reducing them to more stable forms, the antioxidant capacity was measured using in-solution methods. These assays evaluate the antioxidant activity of a sample involving distinct mechanisms of

neutralizing the generated reactive species, including radical-scavenging activity, metal-reducing power, and metal-chelation property. Since the use of a single method often fails to accurately reflect the true antioxidant potential of a complex mixture like an extract, the preference is given to the use of a combination of methods (Rumpf et al., 2023).

In this study, the radical-scavenging activity of Sicilian Sumac extract was measured using the DPPH and ABTS assays. The FRAP assay, on the other hand, was used to assess the metal-reducing capacity of the sample (Benzie & Strain, 1996). The obtained results are presented in Table 1. All three methods enable the estimation of exceptionally high antioxidant activity values. For instance, when comparing the antioxidant activity of Sicilian sumac, as indicated by the ABTS and FRAP values, to that of a more widely recognized and commonly used spice such as saffron, it appears to be higher by 5 and 2 times, respectively (Gaglio et al., 2019). The reducing activity of Sicilian Sumac extract was also measured in terms of antioxidant activity in a cell-based lipid peroxidation model. This system allowed us to consider the ability of antioxidant substances present in the extract to interact with or cross membranes and their stability to cellular metabolism (Wolfe & Liu, 2007). The estimated CAA_{50} value (Table 1) indicates a very high antioxidant activity, being two orders of magnitude lower than that determined by Wolfe and Liu (2007) under the same experimental conditions for extracts rich in polyphenols obtained from various plant matrices of dietary interest (Wolfe & Liu, 2007). The researchers acknowledge the intricacies and multifaceted nature of the cellular model, thus emphasizing that the observed antioxidant activity cannot be exclusively attributed to the intrinsic radical scavenging and reducing properties of the molecules under investigation. Instead, they acknowledge the potential involvement of supplementary mechanisms, including the modulation of gene expression associated with scavenging enzymes or the impact of molecule-protein interactions. Consequently, these complexities underscore the necessity for further exploration to elucidate the comprehensive mechanisms underlying antioxidant activity within cellular environments.

Overall, the obtained results have demonstrated that the high reducing activity shown by the in-solution assays for the hydroalcoholic extract of Sicilian Sumac translates into real antioxidant action in a biological context. Despite the need to verify the digestive stability and bioavailability of antioxidant components present in the extract, it is noteworthy that, considering a volume of gastrointestinal fluids equal to 600 mL, the demonstrated antioxidant activity is achieved at concentrations a thousand times smaller than those achievable in the intestines after the consumption of 1 g of Sumac. This observation opens avenues for further exploration into the potential health benefits of Sicilian Sumac extract at physiologically relevant concentrations, warranting future studies to elucidate its efficacy and mechanisms of action *in vivo*. Additionally, investigating the long-term effects of Sicilian Sumac consumption on oxidative stress-related diseases and exploring its potential applications in functional foods and nutraceuticals represent promising directions for future research endeavors.

3.5. Antiproliferative activity

The consumption of fruits and vegetables has long been associated with a reduced risk of cancer. Experimental data consistently emphasize the cancer-preventive properties found in various phytochemicals (Mir et al., 2023). Despite the extensive documentation of various biological activities in hydrophilic extracts from *Rhus coriaria* fruits, the potential anticancer effects remain poorly explored. Some studies have demonstrated oncostatic actions in *in vivo* models of breast carcinoma (Kubatka et al., 2020) and cytotoxic activity in breast carcinoma cell lines (El Hasasna et al., 2015, 2016; Gabr & Alghadir, 2021; Kubatka et al., 2020). In particular, El Hasasna et al. (2015; 2016) illustrated antiproliferative activity associated with cell cycle arrest and pro-autophagic effects of ethanol extracts from *Rhus coriaria* fruit obtained from Lebanon, associated with ERK1 activation. In a subsequent study, the same authors demonstrated that the same extract at non-cytotoxic concentrations significantly inhibited migration and invasion of MDA-MB231 cells and their adhesion to microvascular endothelial cells. They also documented anti-inflammatory and antiangiogenic actions associated with NF-kB, STAT3, and nitric oxide (NO) pathway modulation.

In our investigation, we examined the antiproliferative activity of a Sicilian *Rhus coriaria* fruit extract against MCF-7 cells (human breast cancer), Caco-2 cells (human colorectal cancer), and, for the first time, against HeLa cells (human cervical cancer) and HepG2 cells (human liver cancer) using the MTT assay (Table 2). The concentrations tested ranged from 200.0 to 20.0 μg DW/mL of cell culture medium. These concentrations, considering a gastrointestinal volume of 600 mL, are significantly lower—ten to two hundred times lower—than those achievable within the intestinal lumen following the consumption of 2–1g of sumac, amounts consistent with its typical culinary usage.

Overall, the sumac extract exhibited concentration-dependent high antiproliferative activity with an average GI_{50} value of 77.88 μg FW/mL, comparable to the IC_{50} values determined by Gabr and Alghadir (2021) for a hydroalcoholic extract from Egyptian sumac, tested in a concentration range of 10–100 μg/mL against ovarian, prostate, and breast cancer cell lines (Gabr & Alghadir, 2021). The GI_{50} value here determined along the four different cell lines ranged from 149.74 ± 8.72 to 31.08 ± 2.25 μg/mL cell medium, highlighting a certain variability in effects depending on the cell type. It is noteworthy that the most substantial growth inhibitory effects were observed against Caco-2 cells, while the least effect was witnessed in HeLa cells.

El Hasasna et al. (2015) reported an IC_{50} value of 433 μg/mL for MCF7 after 72 h of treatment for a hydroalcoholic extract of Lebanese sumac, while greater sensitivity was demonstrated in the other two breast cancer cell lines, with IC_{50} values of 283 and 229 μg/mL for MDA-MB231 and T47D, respectively. In our experimental conditions, the GI_{50} value determined on MCF7 cells is six times lower than the values reported by El Hasasna et al. (2015).

Our results demonstrate that Caco-2 colorectal adenocarcinoma cells are the most sensitive to the antiproliferative action of the extract components. Athamneh et al. (2017) reported antiproliferative actions associated with pro-autophagic and proapoptotic effects of a hydroalcoholic extract of *Rhus coriaria* fruit from Lebanon on the HT29 colorectal cancer cell line, with an IC_{50} at 72 h of 271 μg/mL (Athamneh et al., 2017). The same authors estimated an IC_{50} at 48 h on Caco-2 cells to be 316 μg/mL, an order of magnitude larger than the value determined by us at 72 h for the hydroalcoholic extract of Sicilian *Rhus coriaria*.

While various experimental conditions may contribute to justifying the observed differences in GI_{50} values compared to literature data, it cannot be excluded that a unique phytochemical profile, both qualitative and quantitative, of the *Rhus coriaria* sample selected by us could justify the greater activity observed for the Sicilian sumac extract.

Although the effects on breast, liver, and uterine cancer cells may

Table 2
Antiproliferative activity of Sumac extract against HeLa, HepG2, Caco-2, and MCF-7 tumor cell lines expressed as GI50 values (μg of dry weight per mL of cell medium). Values represented as mean ± SE of three experiments carried out in quadruplicate. Different lowercase letters indicate significant difference at $p \leq 0.05$ as measured by Tukey's multiple.

HeLa	149.74 ± 8.72^{a}
HepG2	58.53 ± 0.53^{c}
Caco-2	31.08 ± 2.25^{d}
MCF-7	77.19 ± 2.87^{b}

have *in vivo* significance only if the blood bioavailability of the extract's phytochemicals is demonstrated, the high sensitivity of colorectal cancer cells to the extract could suggest a real protective potential of consuming this fruit in colorectal cancer prevention. In fact, a local action on intestinal epithelial cells does not depend on the actual bioavailability of the extract components in the blood, and the GI50 value we determined on Caco-2 cells is an order of magnitude lower than the concentrations of *Rhus coriaria* achievable in the intestines after fruit ingestion.

3.6. Antibacterial activity

The antibacterial activity of the PSF extract was assessed against the primary pathogens associated with foodborne bacterial infections, typically utilized for monitoring microbiological food hygiene and safety criteria (Commission, 2005). The results illustrating the inhibitory properties of the PSF extract are presented in Fig. 2. The considerable susceptibility of pathogenic bacteria to sumac extracts derived from various parts of the plant is widely recognized (Perrone et al., 2022). However, the PSF extract evaluated in this study demonstrated exceptionally high antibacterial activity against both Gram-positive strains (*B. cereus*, *L. monocytogenes*, and *St. aureus*) and Gram-negative strains (*E. coli*, *P. aeruginosa*, *S. enteritidis*, and *S. typhimurium*), with inhibition zones ranging from 18.75 to 22.88 mm. These findings underscore the PSF extract's capacity to penetrate the peptidoglycan layer and target the cytoplasmic membrane of Gram-positive bacteria (Rajagopal & Walker, 2017, pp. 1–44) and to destroy the external lipopolysaccharide membrane of the cell wall of Gram-negative bacteria (Rajagopal & Walker, 2017, pp. 1–44). This ability against both Gram-positive and Gram-negative bacteria was previously reported by Mahdavi et al. (2018) for the ethanolic extract of Iranian sumac, while Shahrivari et al. (2024) highlighted the antibacterial activity of sumac water extract only against Gram-positive bacteria. The antibacterial activity of the PSF extract was also quantified in terms of Minimum Inhibitory Concentration (MIC). This microbiological parameter is defined as the lowest concentration of an antimicrobial compound capable of inhibiting microbial growth and serves as a measure of the antibacterial efficacy of a plant-derived compound. All indicator strains exhibited an MIC of 25 mg/mL, except for strain *S. Enteritidis* ATCC13076, which displayed an MIC value of 12.5 mg/mL. A comparable trend was previously observed for the ethanolic extract of flowers and stem bark of sumac cultivated in Iraq. (Nasar-Abbas & Halkman, 2004). The strong antibacterial activity of sumac is probably related to its polyphenol compounds.

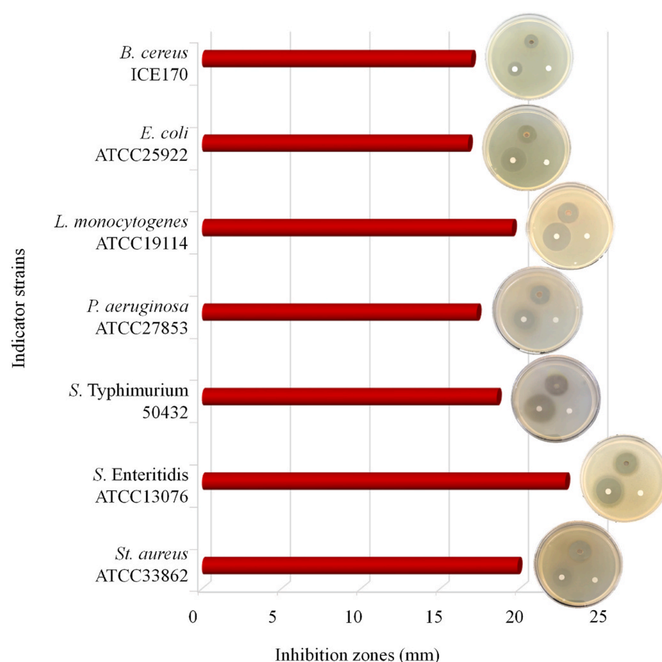

Fig. 2. Inhibitory activity of powdered sumac fruits extract. Each Petri dish is organized in the following way: the upper well is filled with the PSF extract, the paper disk to the left serves as the positive control, and the paper disk on the right-side acts as the negative control.

4. Conclusion

This study revealed that sumac (*Rhus coriaria* L.) fruits, growing wild in Sicily (Italy), represent an interesting source for the extraction of bioactive compounds. Specifically, the phytochemical characterization of the PSF extract showed a very high radical scavenging, antioxidant, and anticancer activity, highlighting its potential protective effects on human health. From a microbiological point of view, the extract was highly effective at low concentrations against the main food-borne pathogenic bacteria used as indicators. These results suggest that Sicilian sumac can be used as natural additive to improve the functional and safety aspects of processed foods.

Despite our findings, toxicological studies on Sumac, particularly focusing on its safety for food applications, are crucial for ensuring consumer health and regulatory compliance. While there is a wealth of research on the medicinal and nutritional properties of Sumac, comprehensive toxicological assessments specific to its use as a food ingredient are relatively scarce in the scientific literature. Existing studies have primarily focused on the safety of Sumac extracts or specific components in vitro and in animal models (Doğan & Çelik, 2016; Sangha et al., 2022; Shafiei et al., 2011; Wu et al., 2018). These studies have generally reported favorable safety profiles, with no significant adverse effects observed at doses commonly used in traditional medicine or dietary supplementation, supporting the potential safety of Sumac for human consumption. However, it's important to note that the safety of Sumac may vary depending on factors such as the specific species, growing conditions, extraction methods, and dosage. Despite these promising findings, further research is needed to comprehensively assess the safety of Sumac for food applications. Future studies should include comprehensive toxicological evaluations, including acute, subchronic, and chronic toxicity assessments in relevant animal models. Additionally, human clinical trials are essential to validate the safety of Sumac consumption in diverse populations and to establish safe dosage levels for dietary use.

CRediT authorship contribution statement

Enrico Viola: Writing – review & editing, Investigation, Formal analysis, Data curation. **Giuseppe Mannino:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Graziella Serio:** Investigation, Formal analysis, Data curation. **Lorenza La Rosa:** Investigation, Formal analysis, Data curation. **Giuliana Garofalo:** Investigation, Formal analysis, Data curation. **Rosario Schicchi:** Writing – review & editing, Investigation, Conceptualization. **Luca Settanni:** Writing – review & editing, Validation, Supervision, Methodology. **Carla Gentile:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104704.

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