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Kombucha Ferments from Savoy Cabbage (*Brassica oleracea* var. *sabauda* L.) By-Products as Bioactive Cosmetics Ingredients with Antioxidant, Anti-Inflammatory and Skin-Protective Activities

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Abstract

Savoy cabbage (*Brassica oleracea* var. *sabauda* L.) is a valuable source of bioactive phytochemicals whose biological activity may be enhanced by kombucha fermentation. This study evaluated the phytochemical profile and biological properties of extracts and kombucha ferments prepared from the core, inner leaves, and outer leaves of savoy cabbage. LC–MS analysis identified metabolites, including gallic acid and chlorogenic acid. Among the tested fractions, the outer leaves displayed the most potent radical-scavenging capacity at 500 µg/mL, reaching 26.8% for ABTS and 22.6% for DPPH. Biocompatibility assessment via Alamar Blue and Neutral Red assays revealed low cytotoxicity, with cell viability exceeding 100% up to concentrations of 250 µg/mL. Fermented samples exhibited stronger anti-inflammatory effects than the corresponding extracts, reducing IL-6 production to 2.66-fold compared to the control. Furthermore, kombucha ferments demonstrated increased antibacterial activity, particularly against *Staphylococcus aureus*. Overall, these results indicate that kombucha fermentation significantly upgrades the bioactivity of savoy cabbage, especially its outer leaf tissue, highlighting its potential application in functional foods and bioactive ingredient formulations.

Keywords: savoy cabbage; *Brassica oleracea* var. *sabauda* L.; kombucha ferments; antioxidants; cytotoxicity assessment; anti-inflammatory activity; antibacterial activity

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1. Introduction

The increasing demand for naturally derived bioactive substances, together with the growing emphasis on sustainable plant resource management, has highlighted the potential of food industry by-products as valuable raw materials. Although substantial amounts of plant biomass are discarded throughout food production, processing, and distribution, these materials often retain a wide range of bioactive constituents that may offer significant health benefits [1,2]. Plant by-products, previously considered of low economic value, such as vegetable cores and outer leaves, are a valuable source of polyphenols, flavonoids, glucosinolates, vitamins, and other secondary metabolites exhibiting a broad spectrum of

biological activity [3]. Reusing these materials supports circular economy principles by minimizing waste and transforming biological resources into high-value products through efficient management [3,4]. A particularly interesting group of raw materials are cruciferous vegetables, where a significant portion of waste biomass consists of outer leaves, cores, and other parts that do not meet commercial requirements despite their high content of bioactive compounds.

Savoy cabbage (*Brassica oleracea* var. *sabauda* L.) is a member of the Brassicaceae family, which includes many vegetable species used in human nutrition, belonging to genera such as *Brassica*, *Raphanus* and *Sinapis*. Vegetables belonging to this family represent a crucial component of a balanced diet due to their high content of diverse biologically active compounds. Of particular significance are glucosinolates and the products derived from their metabolism, which are credited with antioxidant and anti-inflammatory properties, as well as potential chemopreventive effects [5–9]. In addition to its nutritional value, this material is notable for its high content of various biologically active substances, which are credited with antimicrobial, anti-inflammatory and antioxidant properties [10,11]. Its phytochemical composition is marked by a high content of phenolic compounds, primarily represented by flavonoids and hydroxycinnamic acids (including sinapic and chlorogenic acids). In Savoy cabbage, flavonoids occur primarily in the form of glycosidic derivatives of kaempferol and quercetin [11]. The glucosinolates present in this vegetable include, among others, sinigrin [12,13]. This vegetable is also a source of vitamin C and essential minerals, including calcium (Ca), magnesium (Mg), iron (Fe), and potassium (K) [10,12,14,15].

Fermentation is one of the oldest and best-known biological food processing methods, used for centuries to preserve food and improve its sensory properties and nutritional value. In addition to its nutritional benefits, this plant material contains a broad spectrum of biologically active compounds with reported antioxidant, antimicrobial, and anti-inflammatory properties [16,17].

Growing interest in complex microbial consortia has highlighted kombucha, a symbiotic culture of bacteria and yeast (SCOBY), for its capacity to thoroughly process and transform plant materials [18,19]. In addition to providing nutrients, plant-based raw materials serve as an important source of biologically active compounds, which are credited with antioxidant, antimicrobial, and anti-inflammatory properties [18,20,21]. Enzymes produced by the microorganisms present in SCOBY contribute to the partial degradation of plant cell wall components, which can facilitate the release of the bioactive substances contained within them, especially polyphenols, thereby increasing their availability and biological potential [18,19].

SCOBY fermentation is currently seen not only as a method for obtaining functional beverages, but also as an innovative tool for green chemistry and sustainable biotechnology [3]. This process occurs under mild conditions, without the need for high temperatures, aggressive chemical reagents, or energy-intensive technologies, thus achieving a low environmental impact. This process also facilitates the rational use of plant-based raw materials, as well as waste and by-products generated in the food industry, in line with the principles of a circular economy [3,18,22]. The use of kombucha fermentation for the biotransformation of waste materials creates the possibility of obtaining products with an increased content of bioavailable bioactive compounds and high functional value. Savoy cabbage is a suitable raw material for such applications due to the presence of numerous biologically active compounds, including glucosinolates, phenolic compounds, flavonoids, vitamin C, and other phytochemicals. These substances are attributed with potential antioxidant, anti-inflammatory, and antimicrobial properties [23–25]. The biological properties of these compounds make Savoy cabbage-derived materials a potentially valuable source of ingredients for the development of nutraceutical and cosmeceutical products, extending their

potential applications beyond their conventional use as a food product. Moreover, the valorization of Savoy cabbage residues through fermentation may provide an opportunity to recover and biotransform bioactive constituents that would otherwise be lost during processing. Consequently, kombucha fermentation represents a promising strategy for the valorization of Savoy cabbage-derived waste materials and their conversion into products with potentially enhanced functional properties, making this technology a promising development direction for modern food biotechnology, as well as the nutraceutical, cosmeceutical, and pharmaceutical industries [4,26].

This investigation evaluated the phytochemical profile and biological activities of unfermented extracts and kombucha ferments derived from various Savoy cabbage tissues. Key bioactives were profiled using LC-MS. Antioxidant activities were determined through ABTS, DPPH, and FRAP methods, together with intracellular ROS quantification in skin cells. Safety profiles on fibroblasts and keratinocytes were verified using the Alamar Blue and Neutral Red cytotoxicity assays. Furthermore, the preparations were screened for antimicrobial activity against Gram-positive and Gram-negative bacteria, and their anti-inflammatory potential was established via ELISA quantification of specific cytokines.

2. Materials and Methods

2.1. Preparation of Savoy Cabbage Extracts and Kombucha Ferments

Savoy cabbage (*Brassica oleracea* var. *sabauda* L.) was obtained from a local producer. To prepare the samples, the cabbage heads were separated into cores and leaves (comprising both inner and outer leaves). Prior to extraction, the plant tissues were washed, dried and comminuted.

To perform the extraction, 18 g of the prepared mixture containing cores along with inner and outer leaves was added to 600 mL of distilled water. The mixture was continuously stirred with a magnetic stirrer at room temperature for 24 h without heating. Subsequently, the samples underwent 30 min of sonication at ambient temperature using an ultrasonic bath (Digital Ultrasonic Cleaner, Thermo Fisher Scientific, Waltham, MA, USA). Fermentation was performed with a commercially available kombucha starter culture (SCOBY) purchased from Nasza Przyszłość (Szczecinek, Poland). The culture consisted of a symbiotic consortium of acetic acid bacteria (AAB), primarily from the genera *Acetobacter* and *Gluconobacter*, and yeasts belonging to several genera, including *Saccharomyces*, *Zygosaccharomyces*, *Candida*, and *Pichia*.

Fermentation was carried out by transferring 200 mL portions of the extract into sterile 1000 mL beakers. Sucrose (20 g) was added to the media to achieve a final concentration of 10% (w/v), along with the kombucha starter to achieve a final concentration of 10% (v/v). The process was conducted at a constant temperature of 25 °C under aerobic conditions. The beakers were covered with permeable, sterile cotton gauze secured with a rubber band, allowing for oxygen exchange while protecting against contamination. Samples were collected under aseptic conditions at designated time intervals (after 10 and 20 days), with pH measured at each sampling point. pH values ranged from 6.51 to 6.29 for the extracts, from 3.85 to 3.63 for the ferments after 10 days, and from 3.15 to 2.95 for the ferments after 20 days.

The unfermented extract was retained as sample E, while the fermented kombucha samples collected after 10 and 20 days of fermentation were designated as F10 and F20, respectively. The prepared samples were stored at −4 °C.

2.2. Chromatographic Analysis

The qualitative profile of the major secondary metabolites in the analyzed extracts was determined using the UHPLC-DAD-ESI-MS method, which combines liquid chromatog-

raphy with UV–Vis detection and mass spectrometry. Phenolic compounds, including phenolic acids and flavonoids, were identified based on their retention times, UV–Vis spectra, and mass spectrometry data. Quantification of the selected compounds was performed using UHPLC-DAD based on external calibration curves, following the procedure previously described [27].

2.3. Determination of Antioxidant Properties

2.3.1. ABTS Radical Scavenging Assay

Antioxidant properties of extracts and fermentates from Savoy cabbage parts (core, inner and outer leaves) were measured using a modified ABTS method based on Miller et al. [28]. The working ABTS solution was prepared by mixing 7 mM ABTS (Merck KGaA, Darmstadt, Germany) with 2.4 mM potassium persulfate (Warchem, Zakręt, Poland) in a 1:1 ratio and incubating the mixture for 14 h at ambient temperature. The resulting solution was diluted with PBS (Merck KGaA, Darmstadt, Germany) to achieve an absorbance of 1.0 ± 0.04 at 734 nm. Subsequently, 50 µL of each sample (at a concentration of 50–500 µg/mL) was mixed with 150 µL of the ABTS reagent, and the absorbance was measured at 734 nm. Ascorbic acid and Trolox were employed as reference antioxidants, whereas distilled water was utilized as the negative control. Sample blanks were also included to account for the intrinsic absorbance of the samples themselves.

$$\% \text{ ABTS scavenging} = \left(1 - \left(\frac{\text{Abs sample} - \text{Abs background}}{\text{Abs control}}\right)\right) \times 100 \quad (1)$$

2.3.2. DPPH Radical Scavenging Assay

The free radical scavenging capacity of the tested extracts and fermentates was evaluated using the DPPH assay, following the procedure described by Brand-Williams et al. [29] with minor modifications. Samples at final concentrations of 50, 100, 250, and 500 µg/mL were mixed with a 0.4 mM DPPH solution (Merck KGaA, Darmstadt, Germany) in a 1:1 ratio and incubated for 20 min at room temperature in the dark. Absorbance was measured at a wavelength of 517 nm. As positive controls, Trolox (Merck KGaA, Darmstadt, Germany) and ascorbic acid (Merck KGaA, Darmstadt, Germany) were employed, whereas distilled water combined with DPPH reagent acted as the negative control. To subtract the baseline absorbance of the tested samples, blank controls were prepared. Radical scavenging capacity was calculated as a percentage in relation to the negative control. All concentrations were evaluated across three independent experimental trials, each containing three technical replicates.

$$\% \text{ DPPH scavenging} = \frac{\text{Abs control} - (\text{Abs sample} - \text{Abs background})}{\text{Abs control}} \times 100 \quad (2)$$

2.3.3. Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

Antioxidant capacity was determined via a modified FRAP assay based on Benzie and Strain [30]. Aliquots of 20 µL of each extract were combined with 180 µL of working FRAP reagent and incubated for 20 min at room temperature. Absorbance readings were taken at 593 nm. Fe^{3+} reduction capacity was calculated using a Trolox calibration curve (range: 0–1000 µM) and expressed as µM TE/L. Measurements were performed in three independent experiments, each with three technical replicates.

2.3.4. Detection of Intracellular Levels of Reactive Oxygen Species (ROS)

Intracellular reactive oxygen species (ROS) levels in HDF and HaCaT cells were determined using a fluorescent assay with the H_2DCFDA probe (Merck KGaA, Darmstadt,

Germany), following a previously described procedure [31] with minor modifications. Cells were treated for 24 h with the extracts and kombucha ferments at concentrations between 50 and 1000 µg/mL in DMEM (Genos, Łódź, Poland) supplemented with 1% FBS (Genos, Łódź, Poland). After washing, cells were incubated with 10 µM H_2DCFDA probe in serum-free medium and challenged with 500 µM H_2O_2 to induce oxidative stress. Fluorescence readings were recorded at excitation and emission wavelengths of 485 nm and 530 nm, respectively. Unexposed cells served as the negative control, whereas those treated solely with H_2O_2 acted as the positive control. Data from three independent experiments, each conducted in technical triplicate, are presented as relative fluorescence compared to the untreated negative control.

2.4. Cytotoxicity Analysis

2.4.1. Cell Culture

Cytotoxicity evaluation and intracellular reactive oxygen species (ROS) measurements were conducted using human dermal fibroblasts (HDFs; CLS Cell Lines Service, Eppelheim, Germany) and keratinocytes (HaCaTs; CLS Cell Lines Service, Eppelheim, Germany), following the protocol previously described [27].

2.4.2. Alamar Blue (AB) Assay

The viability of HDF and HaCaT cells following exposure to the tested extracts and fermentates was evaluated using the Alamar Blue assay, based on the method described by Page et al. [32] with minor modifications. After a 24 h incubation with the test samples, resazurin (Merck KGaA, Darmstadt, Germany) was added to a final concentration of 60 µM; after an additional 2 h, fluorescence was measured at a wavelength of 570 nm. The results were expressed as a percentage of viability relative to the control and are presented as the mean of three independent experiments, each performed in triplicate.

$$\text{cell viability}[\%] = \frac{(\text{FI sample} - \text{FI background})}{\text{FI control}} \times 100 \quad (3)$$

2.4.3. Neutral Red Uptake Assay

The viability of HDF and HaCaT cells was also evaluated using the Neutral Red Uptake (NRU) assay. Following a 24 h incubation with the test samples, a neutral red solution (40 µg/mL in DMEM) was added, and cells were incubated at 37 °C for 2 h. After incubation, the cells were washed with PBS, followed by dye extraction using an ethanol:acetic acid:water mixture (50:1:49, *v*/*v*/*v*). Absorbance was recorded at 540 nm. Untreated cells were used as the control group. All assays were carried out in three independent experiments, each run in triplicate.

$$\text{cell viability}[\%] = \frac{(\text{Abs sample} - \text{Abs background})}{\text{Abs control}} \times 100 \quad (4)$$

2.5. Assessment of Anti-Inflammatory Activity

The anti-inflammatory activity of Savoy cabbage extracts (E) and preparations fermented for 10 and 20 days (F10 and F20, respectively) was evaluated according to our previously published protocol [33], with some modifications. Briefly, undifferentiated THP-1 (Merck KGaA, Darmstadt, Germany) cells were treated with the test samples (at concentrations of 100 and 250 µg/mL) in the presence of 10 µg/mLLPS ((Merck KGaA, Darmstadt, Germany) for 24 h. Untreated cells and LPS-stimulated cells served as negative and positive controls, respectively, while diclofenac (10 µg/mL) was used as a reference substance. In contrast to the earlier procedure, IL-1β, IL-6, and IL-10 concentrations were quantified in

cell culture supernatants instead of cell lysates. The experiments were performed in three independent replicates, with each replicate consisting of duplicate measurements.

2.6. Determination of Minimum Inhibitory Concentration (MIC) and Bacterial Viability

To establish the antibacterial efficacy of *Brassica oleracea* var. sabauda L. tissues (pith/core, internal foliage, and peripheral leaves), aqueous preparations (E) as well as 10- and 20-day kombucha fermentation broths (designated F10 and F20) were screened. The experimental setup relied on a microdilution assay combined with resazurin viability staining, modified from methodologies detailed elsewhere [24,25,32–35].

Testing targeted five documented bacterial isolates: two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 35032) and three Gram-positive (*S. aureus* ATCC BAA-2312™, *S. capitis* ATCC 146™, and *M. luteus* ATCC 10240™). Prior to testing, the isolates were regenerated in TSA medium (Argenta, Poznań, Poland) at 37 ± 1 °C for 18–24 h. Cell suspensions were prepared in MHB (Argenta, Poznań, Poland) by standardizing the optical density (OD_{625} = 0.080–0.100) and subsequently diluted with broth to ensure an assay titer close to 1.5×10^5 CFU/mL. The assay was carried out in sterile flat-bottom 96-well plates (Googlab Scientific, Rokocin, Poland). The highest concentration (100 µL of 10 mg/mL stock) was loaded into the first well and diluted serially 1:1 in MHB to generate a test range of 39 to 5000 µg/mL.

Bacterial suspensions (25 µL per well) were inoculated and incubated under aerobic conditions for 20 h at 37 °C. To assess viable cell counts, 50 µL of 0.05% (*w*/*v*) resazurin reagent in water was added per reaction. After 30 min of dark incubation at 37 °C, fluorometric signals were measured (Ex/Em = 530/595 nm) on a Synergy multi-mode reader (BioTek, Agilent Technologies, Santa Clara, CA, USA). Several controls were included in each experimental run. Bacterial growth controls contained microorganisms cultured in MHB without the tested preparations, whereas sterility controls consisted of MHB alone. Wells containing MHB and resazurin served as resazurin controls. In addition, sample blanks contained the respective extracts or ferments in MHB with resazurin but without bacterial cells. These blanks were used to account for the intrinsic fluorescence of the plant-derived preparations and for any non-biological reduction of resazurin. As positive controls, ciprofloxacin was applied against Gram-negative bacteria (*E. coli* and *P. aeruginosa*), whereas gentamicin served as the reference standard for Gram-positive organisms (*S. aureus*, *S. capitis*, and *M. luteus*).

Before analysis, all extracts and ferments were dissolved in PBS. The pH of the resulting stock solutions was close to neutral, with values of 7.09–7.12 for the extracts, 6.90–6.95 for F10, and 6.85–6.92 for F20. These values indicated that the antibacterial activity observed in the assay was unlikely to be attributable solely to sample acidity.

The MIC was defined as the lowest concentration of the tested preparation that completely inhibited bacterial metabolic activity, demonstrated by the lack of resazurin reduction relative to the bacterial growth control. All experiments were carried out independently in triplicate.

2.7. Transepidermal Water Loss (TEWL) and Skin Hydration Measurements

Skin hydration and transepidermal water loss (TEWL) were determined using the Corneometer® CM825 and Tewameter® TM300 probes, respectively. Both devices were integrated into the MPA measurement system (Courage + Khazaka Electronic, Cologne, Germany). The room conditions were a temperature of 20 °C and relative humidity between 40 and 60. The study was conducted on a group of 10 women aged 35–65 with a college education, living in a city with a population of 150,000–200,000. Exclusion criteria were: pregnant or breastfeeding women; cancer, chemotherapy, or radiation therapy; fever or an active systemic infection (e.g., a cold); individuals taking antiallergic medications or using

antiallergic treatments; individuals who had recently been exposed to intense sunlight (UV radiation). On the forearm of each subject, 2 × 2 cm test zones were marked and treated with 0.2 mL of the tested cabbage enzymes and extracts. The application sites for the test formulations were selected on the palmar side of the forearms, in an area where the skin condition was as uniform as possible, without visible skin lesions, irritation, or damage. To minimize the impact of local variations in skin condition, all measurements were performed on corresponding, predesignated areas of the forearm, and the test formulations were assigned to specific sites according to a predetermined scheme. Prior to the experimental procedures, participants underwent a 15 min acclimatization period to minimize potential effects of preceding physical activity on peripheral blood circulation. Written informed consent was obtained from all participants before their inclusion in the study. All procedures were performed in accordance with Regulation (EC) No 1223/2009 of the European Parliament and of the Council, the Cosmetics Europe guidelines for the assessment of skin compatibility (1997) and cosmetic product efficacy (2008), and the ethical principles established in the World Medical Association (WMA) Declaration of Helsinki concerning research involving human participants.

2.8. Statistical Analysis

The results are presented as mean values ± standard deviation (SD), based on three independent experiments. Statistical significance was assessed using the one-way analysis of variance (ANOVA), followed by Dunnett's and Tukey's post hoc multiple comparison tests. Differences between the experimental groups and the control were considered statistically significant at the following levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. All statistical analyses were performed using the GraphPad Prism software (version 8.4.3; GraphPad Software, Inc., San Diego, CA, USA).

3. Results and Discussion

3.1. Chromatographic Analysis

UHPLC-DAD-MS analysis was performed to establish the phytochemical profiles of extracts prepared from different anatomical parts of Savoy cabbage, including the core, inner leaves, and outer leaves, as well as the kombucha starter itself. The profiles were assessed at the start of fermentation (day 0), and subsequently on days 10 and 20. As shown in the representative chromatograms (Figure S1), the chromatographic profiles of the cabbage extracts at the beginning of fermentation were relatively simple and characterized by the presence of only a limited number of phenolic constituents. These included chlorogenic acid (0.02, 0.01, and 0.05 µg/mL in the core, inner leaf, and outer leaf extracts, respectively) and a flavonoid with m/z 609 (estimated molecular formula $C_{27}H_{30}O_{16}$), detected in trace levels. Previously, the occurrence of this flavonoid was likewise noted in Savoy cabbage [3]. Additionally, trace quantities of p-coumaric acid isomers (0.05 µg/mL) and kaempferol hexoside (0.01 µg/mL) were detected exclusively within the extract from outer leaves. In contrast, the phytochemical profile of the kombucha starter was considerably more complex and was consistent with our previous findings. It comprised gallic acid (0.93 µg/mL), galloylquinic acids (0.65 µg/mL), chlorogenic acids (0.16 µg/mL), catechin/epicatechin (0.20 mg/g DW), coumaroylquinic acid isomers (0.49 µg/mL), and flavonoids (1.12 µg/mL), represented by quercetin, kaempferol, and apigenin derivatives. During fermentation, no new phenolic metabolites were detected. However, substantial quantitative changes in the concentrations of individual compounds were observed (Table 1, Figure S2). The most pronounced changes occurred during the first 10 days of fermentation, whereas the rate of metabolite accumulation generally declined between days 10 and 20. Interestingly, the final concentrations of several phenolic compounds depended on the part of the cabbage used for fermentation. For

example, the highest accumulation of chlorogenic acids was observed in ferments prepared from outer leaves, reaching 0.65 µg/mL after 20 days, which corresponds to a 306% increase compared with the kombucha starter. Similarly, the accumulation of gallic acid was also greatest in the outer leaf ferment, where its concentration increased from 0.93 to 2.06 µg/mL (+122%). Flavonoids exhibited a more diverse response to fermentation than phenolic acids. While the concentrations of most kaempferol, quercetin, and apigenin derivatives increased during fermentation, the extent of accumulation depended on both the fermentation time and the cabbage tissue used. Among the identified flavonoids, quercetin 3-O-rutinoside showed the most pronounced increase, reaching up to a 4.6-fold higher concentration than that detected in the kombucha starter. In contrast, quercetin 3-O-glucoside and kaempferol hexoside gradually decreased during fermentation in most samples.

Table 1. Quantitative phytochemical analysis of Savoy cabbage (µg/mL).

Compound	Savoy Cabbage Core		Savoy Cabbage Inner Leaf		Savoy Cabbage Outer Leaf	
	F10	F20	F10	F20	F10	F20
Gallic acid *	1.67 ± 0.12 ^{b}	1.97 ± 0.12 ^{a}	1.63 ± 0.11 ^{b}	1.78 ± 0.11 ^{b}	1.83 ± 0.10 ^{b}	2.06 ± 0.13 ^{a}
Galloylquinic acids	1.11 ± 0.08 ^{c}	1.30 ± 0.08 ^{a}	1.12 ± 0.07 ^{c}	1.27 ± 0.09 ^{a}	1.22 ± 0.09 ^{b}	1.28 ± 0.08 ^{a}
Chlorogenic acids *	0.30 ± 0.02 ^{c}	0.37 ± 0.03 ^{b}	0.30 ± 0.02 ^{c}	0.32 ± 0.02 ^{c}	0.57 ± 0.04 ^{a}	0.65 ± 0.04 ^{a}
Catechin/epicatechin *	0.46 ± 0.03 ^{b}	0.58 ± 0.04 ^{a}	0.49 ± 0.03 ^{b}	0.57 ± 0.04 ^{a}	0.51 ± 0.04 ^{b}	0.57 ± 0.03 ^{a}
p-Coumaroylquinic acid isomers *	0.78 ± 0.06 ^{b}	0.93 ± 0.06 ^{a}	0.75 ± 0.04 ^{b}	0.79 ± 0.05 ^{b}	0.93 ± 0.06 ^{a}	0.97 ± 0.06 ^{a}
Quercetin 3-O-glucoside *	0.03 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}	0.06 ± 0.00 ^{a}	0.05 ± 0.00 ^{a}	0.02 ± 0.00 ^{b}
Kaempferol derivative *m*/*z* 593	0.42 ± 0.03 ^{c}	0.59 ± 0.04 ^{a}	0.44 ± 0.03 ^{bc}	0.52 ± 0.03 ^{b}	0.50 ± 0.04 ^{b}	0.55 ± 0.04 ^{ab}
Kaempferol hexoside	0.04 ± 0.00 ^{b}	0.05 ± 0.00 ^{b}	0.04 ± 0.00 ^{b}	0.06 ± 0.00 ^{a}	0.07 ± 0.00 ^{a}	0.03 ± 0.00 ^{b}
Kaempferol derivative *m*/*z* 755	0.23 ± 0.02 ^{c}	0.31 ± 0.02 ^{a}	0.22 ± 0.02 ^{c}	0.26 ± 0.02 ^{b}	0.28 ± 0.02 ^{b}	0.31 ± 0.02 ^{a}
Apigenin derivative *m*/*z* 577	0.26 ± 0.02 ^{c}	0.36 ± 0.02 ^{a}	0.25 ± 0.02 ^{c}	0.29 ± 0.02 ^{b}	0.31 ± 0.02 ^{b}	0.34 ± 0.03 ^{ab}
Flavonoid *m*/*z* 563	0.34 ± 0.02 ^{c}	0.43 ± 0.03 ^{ab}	0.37 ± 0.03 ^{bc}	0.41 ± 0.03 ^{b}	0.41 ± 0.02 ^{b}	0.48 ± 0.03 ^{a}
Quercetin 3-O-rutinoside *	0.53 ± 0.04 ^{b}	0.69 ± 0.04 ^{a}	0.55 ± 0.04 ^{b}	0.73 ± 0.05 ^{a}	0.50 ± 0.04 ^{b}	0.56 ± 0.03 ^{b}
Kaempferol derivative *m*/*z* 609	0.02 ± 0.00 ^{c}	0.02 ± 0.00 ^{c}	0.02 ± 0.00 ^{c}	0.03 ± 0.00 ^{c}	0.22 ± 0.02 ^{a}	0.26 ± 0.02 ^{a}
Quercetin derivative *m*/*z* 771	0.11 ± 0.01 ^{a}	0.13 ± 0.01 ^{a}	0.09 ± 0.01 ^{b}	0.11 ± 0.01 ^{a}	0.07 ± 0.00 ^{c}	0.10 ± 0.01 ^{b}

Values are expressed as mean ± standard deviation (SD, $n = 3$). Different superscript letters within the same row indicate statistically significant differences among samples according to the one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). F10 and F20 denote fermentation for 10 and 20 days, respectively. * Quantified using an authentic external standard.

3.2. Assessment of Antioxidant Activity

3.2.1. Antioxidant Potential Measured by ABTS, DPPH and FRAP Assays

The antioxidant potential of Savoy cabbage extracts and the material remaining after fermentation was determined using three complementary spectrophotometric methods: ABTS, DPPH, and FRAP. The use of multiple analytical approaches enabled a more comprehensive characterization of antioxidant capacity, as bioactive compounds may exert their antioxidant effects through distinct mechanisms. The ABTS assay is based on the reduction of the stable ABTS radical cation ($ABTS^{\bullet+}$), which exhibits a characteristic blue-green color. As antioxidants are introduced, the radical cation undergoes reduction, which progressively diminishes the color intensity of the solution [36]. The DPPH method, on the other hand, utilizes the stable DPPH• radical with an intense purple color. Antioxidant compounds present in the samples can donate an electron or a hydrogen atom, leading to radical reduction and a decrease in absorbance [29]. Building upon traditional anti-radical assays, the FRAP method enables the evaluation of a sample's reduction potential. It measures how effectively antioxidant compounds convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), where a stronger reducing power directly correlates with higher antioxidant capacity [30].

Four concentrations of the test samples were selected for the analysis: 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL. The results of the ABTS and DPPH assays were

expressed as a percentage of free radical scavenging capacity, while the FRAP method was used to evaluate the reducing properties of the analyzed material. The findings confirmed that both the cabbage extracts and their fermentation products possess notable antioxidant capacity. In the case of ferments, changes occurring during fermentation may also be important, as they can affect the availability and biological activity of antioxidant compounds.

In the ABTS experiment, we observed a very clear trend of antioxidant potential growth with concentration growth. A positive effect of fermentation in most cases was also observed, mainly in higher concentrations such as 250 and 500 µg/mL. At the highest concentration, an increase of about 100% between the extract and F20 ferment was observed; at the lowest concentration though, the effect of fermentation was weak. Comparison between plant parts shows the best results for outer leaves over inner leaves and core. The highest overall results were observed for Savoy cabbage outer leaves for the F20 ferment at the highest concentration (Figure 1, Table 2).

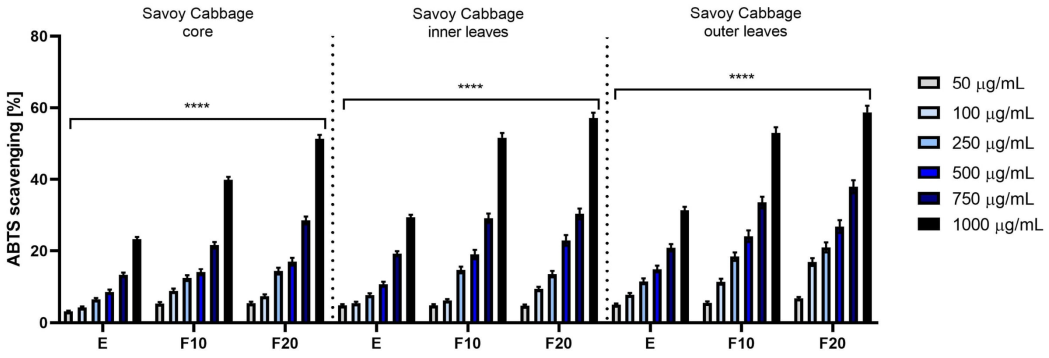


Figure 1. The ABTS radical scavenging capacity was determined for extracts of Savoy cabbage (E) and for samples fermented for 10 (F10) and 20 days (F20), prepared from the core, inner leaves, and outer leaves of the Savoy cabbage. The analyses were conducted using concentrations in the range of 50–1000 µg/mL. The results are presented as the arithmetic mean ± standard deviation (SD), calculated based on three independent experiments, each performed in triplicate. **** $p < 0.0001$.

In the DPPH experiment, we observed a very clear trend of antioxidant potential growth with concentration growth. This is true with no exceptions for all the cases, although results for the Savoy cabbage core for the two highest concentrations remain on a similar level. The effect of fermentation was positive in most cases, mainly at higher concentrations such as 250 and 500 µg/mL. At the highest concentration, an increase of about 100% between the extract and F20 ferment was observed, while at lower concentration, this increase was not as intense as at moderate concentrations, or even weak at the lowest concentration. Comparison between plant parts shows the best results for outer leaves over inner leaves and core. The highest overall results were observed for Savoy Cabbage outer leaves for the F20 ferment at the highest concentration (Figure 2, Table 2).

In the FRAP experiment, we observed a trend of antioxidant potential growth with concentration growth, although this growth in some cases was not too big, like for the extract from the Savoy cabbage core, for instance. At two lowest concentrations, in most cases, results remain at a similar level, but at higher concentrations, growth with concentration is clear. The effect of fermentation was neutral for the two lowest concentrations, but at the highest concentration, it was notable and positive. Comparison between plant parts shows the best results for outer leaves over inner leaves and core. The highest overall results were observed for Savoy Cabbage outer leaves for the F20 ferment at the highest concentration (Figure 3).

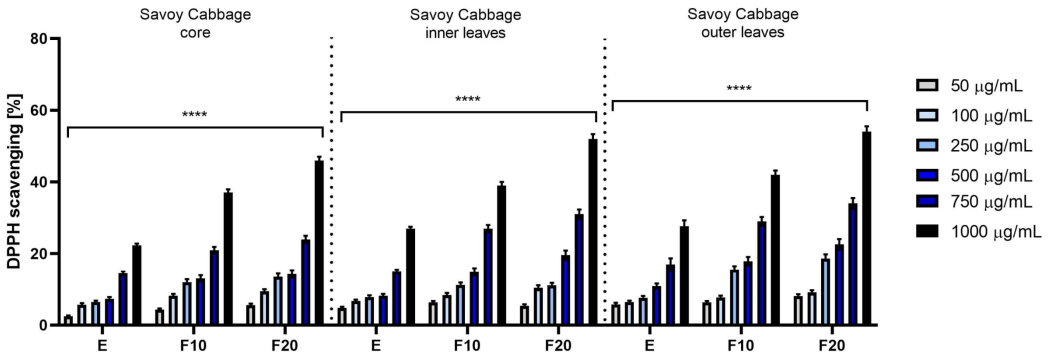


Figure 2. DPPH radical scavenging capacity of unfermented extracts (E) and 10- and 20-day fermentates (F10, F20) derived from Savoy cabbage core, inner, and outer leaves The analyses were conducted using concentrations in the range of 50–1000 µg/mL. The results are presented as the arithmetic mean ± standard deviation (SD), calculated based on three independent experiments, each performed in triplicate. **** $p < 0.0001$.

Table 2. Half-maximal inhibitory concentration (IC_{50}) values for ABTS and DPPH radical scavenging activity of Savoy cabbage extracts and fermentation-derived samples. Results are reported as mean ± standard deviation (SD) from three independent replicates.

	IC50 [µg/mL]								
	Savoy Cabbage Core			**Savoy Cabbage Inner Leaves**			**Savoy Cabbage Outer Leaves**		
	E	**F10**	**F20**	**E**	**F10**	**F20**	**E**	**F10**	**F20**
ABTS	>1000	>1000	952	>1000	944	921	>1000	938	902
DPPH	>1000	>1000	>1000	>1000	>1000	941	>1000	>1000	929

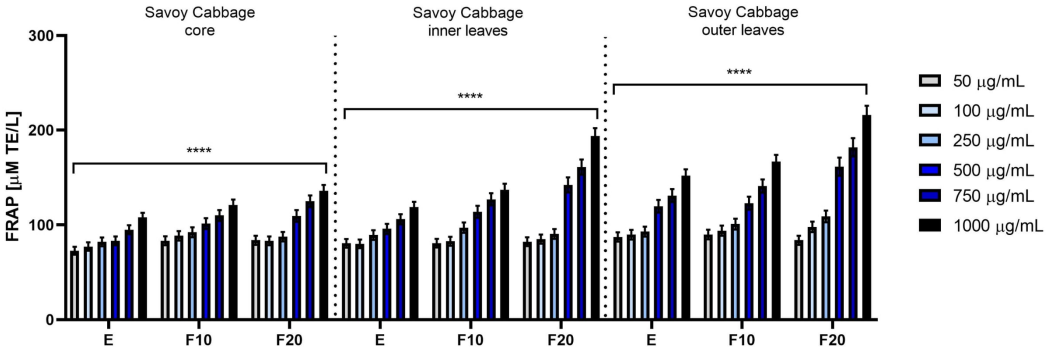


Figure 3. The iron reducing capacity was determined for extracts of Savoy cabbage (E) and for samples fermented for 10 (F10) and 20 days (F20), prepared from the core, inner leaves, and outer leaves of the Savoy cabbage. The analyses were conducted using concentrations in the range of 50–1000 µg/mL. The results are presented as the arithmetic mean ± standard deviation (SD), calculated based on three independent experiments, each performed in triplicate. **** $p < 0.0001$.

These results demonstrate that Savoy cabbage is a valuable reservoir of biologically active substances with strong antioxidant properties. The antioxidant potential of the analyzed samples can be primarily attributed to their content of phenolic compounds, ascorbic acid, carotenoids, and glucosinolates [11,37]. Variations in antioxidant activity among the samples may stem both from differences in the content of these substances and from interactions occurring between the individual components. Ascorbic acid, polyphenols, carotenoids, tocopherols, and compounds resulting from the metabolic transformation of glucosinolates may all play a significant role. Therefore, different results in DPPH, ABTS, or FRAP tests may not necessarily be due solely to the total phenolic content, but also to

the presence of specific metabolite fractions with different reactivity towards radicals and metal ions [38]. The fermentation process may also be a significant factor influencing the antioxidant properties of Savoy cabbage. Microbial fermentation can modify the phytochemical composition of the raw material through the hydrolysis of bound compounds, the breakdown of cellular structures, the release of phenols, and the formation of new metabolites with lower molecular weight [3,12,39,40]. Consequently, compounds present in the plant material may become more readily available, and their ability to neutralize reactive oxygen species may be enhanced. A similar mechanism has been described for fermented white cabbage, where a gradual increase in antioxidant activity, determined by the ABTS and DPPH methods, was observed during fermentation [3].

The results obtained for Savoy cabbage can also be interpreted in the context of studies on the effect of lactic acid fermentation on the bioactive composition of cabbage. Researchers [41] demonstrated that fermentation of white cabbage led to an increase in antioxidant activity, which the authors attributed, among other things, to changes occurring during the grinding and fermentation of the raw material. Martínez-Villaluenga et al. [42] reported that fermentation conditions can significantly affect the content of glucosinolates and ascorbic acid. This indicates that the effect of both kombucha and lactic acid fermentation is not uniform and depends on many factors. The obtained results are consistent with previous reports on *Brassica* vegetables but also point to the need for further research.

These results confirm that both the leaves and the inner parts of Savoy cabbage can be considered a source of active ingredients. This is also important from the perspective of sustainable use of plant materials, where parts that are less attractive technologically or consumer-wise can constitute valuable raw materials.

3.2.2. Intracellular ROS Levels in Skin Cells

In the next phase of the investigation, we evaluated how the Savoy cabbage extracts and their corresponding fermentates influence intracellular reactive oxygen species (ROS) levels in skin cell models. Excessive accumulation of ROS leads to oxidative stress, which can result in damage to lipids, proteins, and DNA, disruption of intracellular homeostasis, and the exacerbation of inflammatory responses and skin aging processes. The experiments were conducted using HaCaT keratinocytes and HDFs, employing the fluorescent probe H_2DCFDA. Upon entering the cells, the probe undergoes a chemical transformation; its subsequent oxidation by reactive oxygen species results in the formation of a fluorescent compound, DCF. The signal generated by this compound allows for the assessment of ROS levels within the cells. Fluorescence intensity allows for a quantitative assessment of oxidative stress levels [43].

In the ROS HDF experiment, in all the cases, we observed a clear trend as intracellular ROS fold lowered with increased concentration. Moreover, in all the cases, results were close to or lower compared to the positive control, in some cases in a significant way. The effect of fermentation was definitely positive. For each plant part, the overall results show lowering as fermentation time grows. In each case, the best results for any given plant part were always obtained for the F20 ferment at the highest concentration. Comparison between various parts of plant showed that results obtained for outer leaves are the most preferable. When we considered the F10 and F20 ferments analyzed at higher concentrations, we observed that the drop of the intracellular fold was clearly more significant for outer leaves compared to the other plant parts (Figure 4).

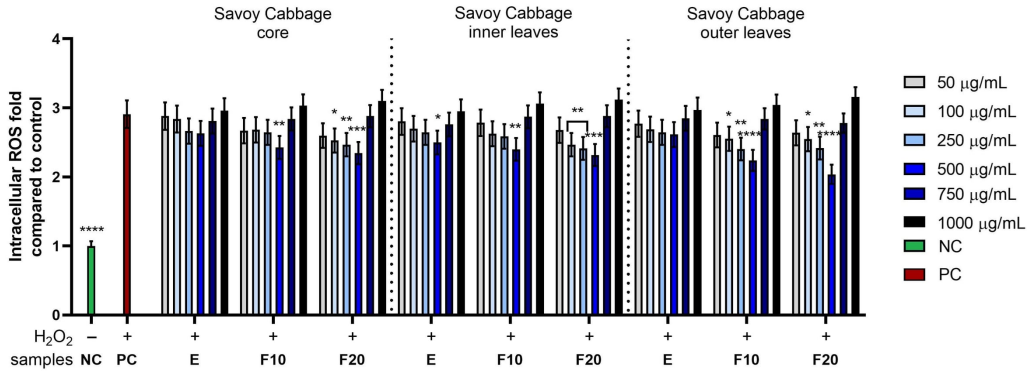


Figure 4. The effect of extracts (E) and fermentation products obtained after 10 (F10) and 20 days (F20), prepared from the core, inner leaves, and outer leaves of Savoy cabbage, on the intracellular level of reactive oxygen species (ROS) in human dermal fibroblasts (HDFs) was evaluated. The analysis was conducted using concentrations ranging from 50 to 1000 µg/ml. Untreated cells maintained in culture medium served as the negative control (NC), while cells treated with H_2O_2 functioned as the positive control (PC). Results represent the arithmetic mean ± standard deviation (SD) from three biological replicates, each conducted in triplicate. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

In the ROS HaCaT experiment, in all the cases, we observe a clear trend as intracellular ROS fold lowered with increased concentration. Moreover, in all the cases, results were lower compared to the positive control, in some cases in a significant way. We observed that fermentation has a positive effect. For each plant part, at higher concentrations, results showed a clear lowering as fermentation time grows. In each case, the best results for any given part of a plant were always obtained for the F20 ferment at the highest concentration. Comparison of various plant parts showed that outer leaves provide the best results. Results for outer leaves at higher concentrations, especially for ferments, were better than for other parts. The lowest overall numbers were registered for outer leaves F10 and F20 ferments at the highest concentration (Figure 5).

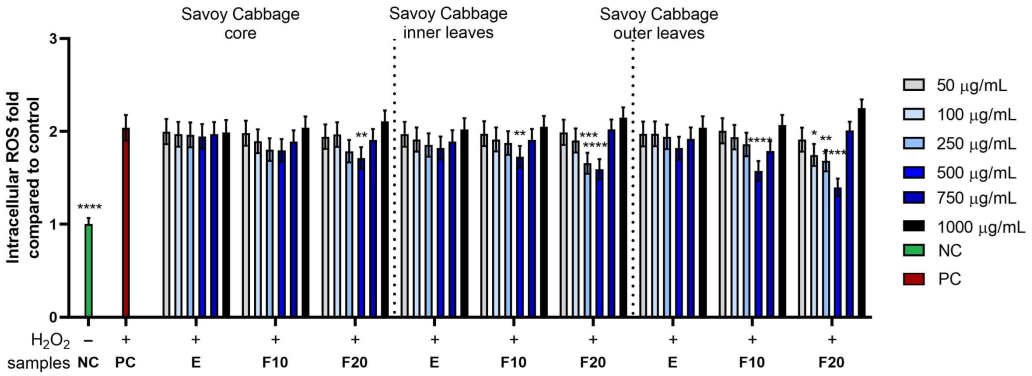


Figure 5. The effect of extracts (E) and fermentation products obtained after 10 (F10) and 20 days (F20), prepared from the core, inner leaves, and outer leaves of Savoy cabbage, on the intracellular level of reactive oxygen species (ROS) in keratinocytes (HaCaTs) was evaluated. The analysis was conducted using concentrations ranging from 50 to 1000 µg/ml. Untreated cells maintained in culture medium served as the negative control (NC), while cells treated with H_2O_2 functioned as the positive control (PC). Results represent the arithmetic mean ± standard deviation (SD) from three biological replicates, each conducted in triplicate. **** $p < 0.0001$, *** $p = 0.0001$, ** $p < 0.001$, * $p = 0.051$.

Determining the intracellular levels of reactive oxygen species (ROS) in HaCaT keratinocytes and human dermal fibroblasts (HDFs) made it possible to assess the potential protective properties of extracts and ferments derived from the leaves and cores of Savoy cabbage, with respect to these primary skin cell types. The observed reduction in ROS

generation suggests potential cytoprotective properties. Furthermore, previous studies on fermented plant extracts indicate that kombucha fermentation may increase antioxidant activity and protect skin cells exposed to oxidative stress [38,43,44]. Similar observations have been reported for ferments obtained from white and red cabbage by-products, where their biological activity and safety in skin cells were analyzed [3].

In summary, the determination of ROS in skin cells allows for a better assessment of the biological significance of the antioxidant activity of Savoy cabbage extracts and ferments. These findings complement the data obtained from the ABTS, DPPH, and FRAP assays, making it possible to evaluate whether the tested preparations possess the ability to directly reduce oxidative stress in vitro, using a cellular model. Ferments from the leaves and core of Savoy cabbage may therefore constitute an interesting material for further research on components with cytoprotective and dermoprotective potential.

3.3. Cytotoxicity Analysis

In order to assess the impact of extracts and fermentates obtained from Savoy cabbage on the viability of skin cells, an analysis of their potential impact on the tested cells was carried out. Two complementary cytotoxicity assays were used: Alamar Blue (AB) and Neutral Red (NR). The Alamar Blue method was employed to quantify cellular metabolism, evaluating the enzymatic conversion of resazurin into fluorescent resorufin within viable cells. This method is particularly useful in assessing early metabolic changes that may precede more advanced cellular damage. This evaluation was complemented by the Neutral Red assay, which measures lysosomal membrane integrity and the capacity of viable cells to incorporate and retain the dye within their lysosomes. Reduced neutral red accumulation may indicate impaired lysosomal function, structural damage, or a decrease in the number of cells capable of functioning properly. Application of both assays to fibroblast and keratinocyte cell models allows for the distinction between whether the observed changes are primarily related to cellular metabolism or are associated with damage to intracellular structures [45,46]. The analysis included samples obtained from cabbage leaves and core, used in the concentration range of 50, 100, 250 and 500 µg/mL.

In the AB HDF experiment, in most cases, we observed that with increased concentration cell viability first grows over the level of control, but then for the highest concentrations drops down. Fermentation process results showed decreasing observed values, as in the case of core and outer leaves, or has minimal effect like in the case of inner leaves. Comparison between various parts of plant showed that for core and inner leaves the obtained results were very similar results, while for outer leaves results were slightly lower. The highest overall activity was observed in the case of extracts obtained from the core and outer leaves of Savoy cabbage. In both cases, maximum values were recorded at a concentration of 100 µg/mL (Figure 6).

In the AB HaCaT experiment, cell viability at the highest concentration was lower than that of the control under most conditions. At low and moderate concentrations, cell viability remained above the control level. For a given fermentation time and anatomical fraction, the highest cell viability was generally observed at moderate concentrations (100 or 250 µg/mL). The highest cell viability overall was recorded for the following variants, all at the same significance level: core extract at 100 µg/mL, inner leaf F10 ferment at 100 and 250 µg/mL, and outer leaf extract at 100 and 250 µg/mL. Fermentation time also affected the response. At higher concentrations, cell viability decreased slightly with increasing fermentation time, whereas no effect of fermentation time was observed at the lowest concentrations. At moderate concentrations, the response differed according to the anatomical fraction. The highest cell viability was observed for extracts from the core and outer leaves, whereas F10 and F20 ferments produced the highest cell viability in inner leaves (Figure 7).

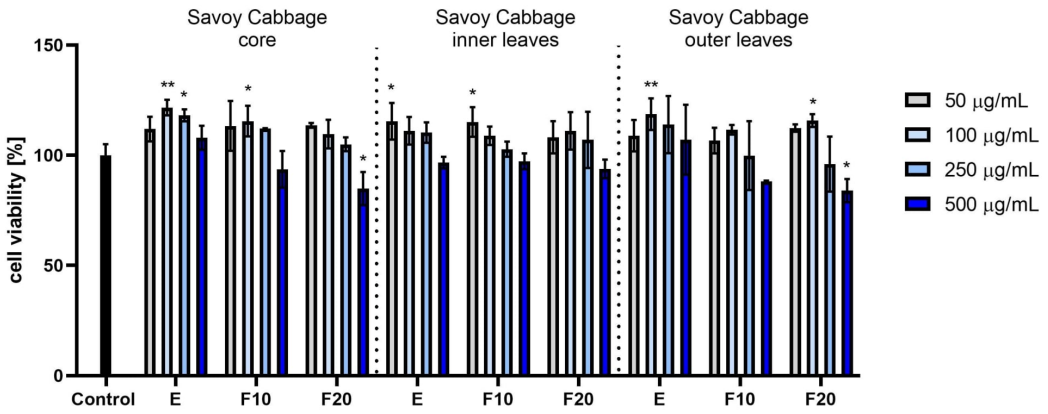


Figure 6. Effects of increasing concentrations (50–500 µg/mL) of Savoy cabbage extracts (E) and kombucha ferments collected after 10 and 20 days (F10 and F20, respectively), obtained from the core, inner leaves, and outer leaves, on dermal fibroblast viability evaluated using the Alamar Blue assay. Data are expressed as the mean ± standard deviation (SD) of three independent experiments performed in triplicate. ** $p < 0.01$, * $p < 0.05$.

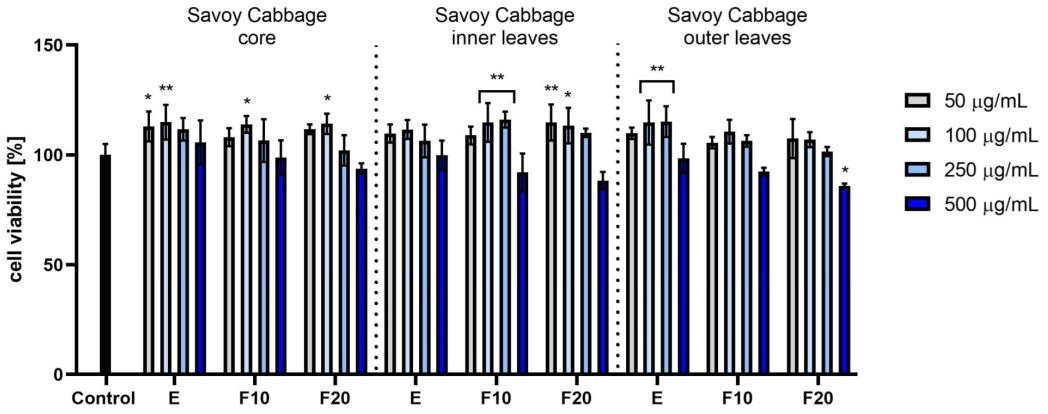


Figure 7. Effects of increasing concentrations (50–500 µg/mL) of Savoy cabbage extracts (E) and kombucha ferments collected after 10 and 20 days (F10 and F20, respectively), obtained from the core, inner leaves, and outer leaves, on keratinocytes viability evaluated using the Alamar Blue assay. Data are expressed as the mean ± standard deviation (SD) of three independent experiments performed in triplicate.** $p < 0.01$, * $p < 0.05$.

In the NR HDF experiment, it was observed that, at the highest concentration, in most cases, cell viability was lower than that of the control, only the extract from the core gave a result on similar level. All other measurements, for lower and moderate concentrations, remained on a similar or even higher level than that of the control. Looking for optimal concentration for given fermentation time and plant part leads to conclusion that, in most cases, the most preferable were moderate concentrations of 100 or 250 µg/mL. Overall, optimal performance was recorded for the core F10 ferment at 100 and 250 µg/mL, and the outer leaf F20 ferment at 100 µg/mL. Another observed effect was a result of fermentation time. It was observed that at higher concentrations cell viability decreases slightly with increased fermentation time. In the lowest concentrations a slight increase or no change with fermentation time was observed. In moderate concentrations, the result depended on which part of the plant is considered, the core and outer leaves; the F10 ferment gave the highest results, while for inner leaves, the results were very close independently on fermentation time (Figure 8).

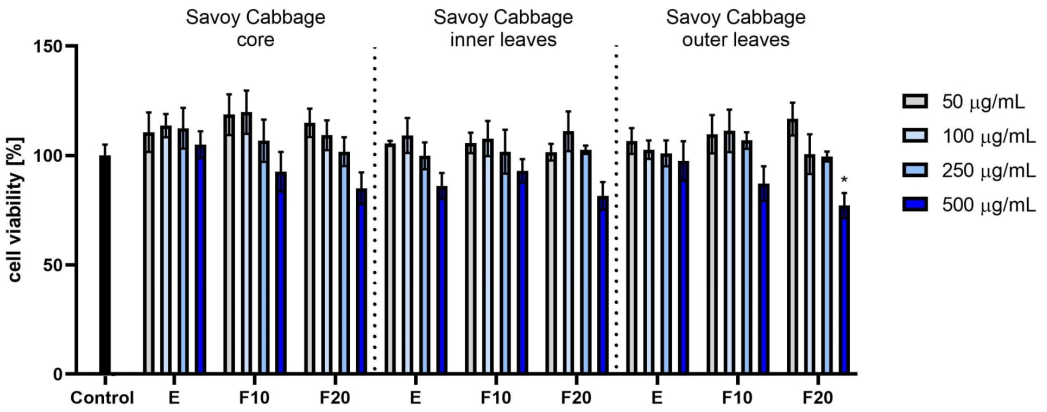


Figure 8. Impact of increasing concentrations (50–500 µg/mL) of Savoy cabbage extracts (E) and kombucha ferments (F10 and F20), prepared from the core, inner leaves, and outer leaves, on dermal fibroblast cell viability assessed via the Neutral Red uptake assay. Values represent the mean ± SD derived from three independent experiments performed in triplicate for each treatment group. * $p = 0.0253$.

In the NR HaCaT experiment, it was observed that, at the highest concentration, in most cases, cell viability was lower than that of the control; only the extract from the core gave a result on similar level. Other measurements, for lower and moderate concentrations, remained on similar or higher level compared to the control. The best results overall were obtained for: core extract in concentration of 250 µg/mL, core F10 ferment in concentration of 100 µg/mL, inner leaves F20 ferment in concentrations of 100 µg/mL, all at the same significance level. The impact of fermentation time depends on the concentration. It was observed that at higher concentrations cell viability decreases with increased fermentation time. At the lowest concentration, no change or just a slight increase due to fermentation time was observed. In moderate concentrations, in the case of the core, the effect was weak; in the case of inner leaves, an increase was observed; in the case of outer leaves, there was no change observed (Figure 9).

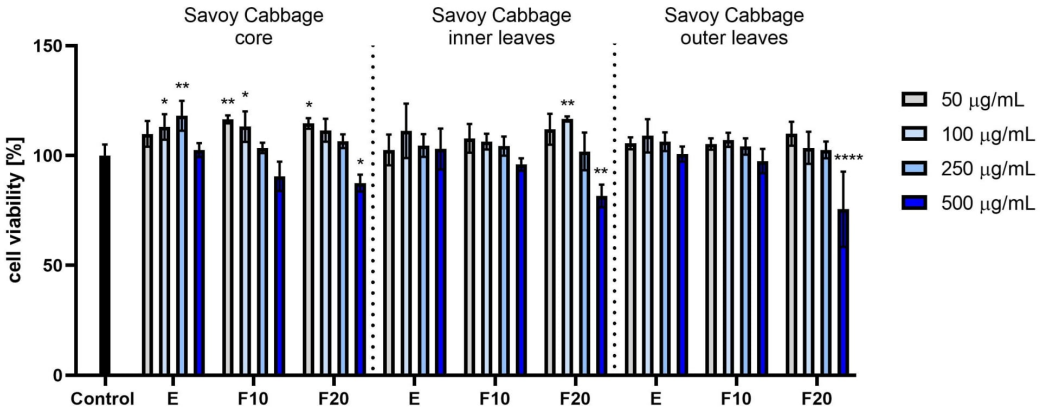


Figure 9. Impact of increasing concentrations (50–500 µg/mL) of Savoy cabbage extracts (E) and kombucha ferments (F10 and F20), prepared from the core, inner leaves, and outer leaves, on keratinocytes cell viability assessed via the Neutral Red uptake assay. Values represent the mean ± SD derived from three independent experiments performed in triplicate for each treatment group. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

Evaluating the cytotoxic profile of extracts and fermentates derived from Savoy cabbage leaves and core constitutes a crucial step in determining their biological safety. The use of HaCaT keratinocytes and human dermal fibroblasts (HDFs) provides a relevant in vitro

model for investigating plant-derived materials intended for incorporation into cosmetic formulations, dermoprotective products, and functional preparations [1,47].

The absence of a significant decrease in cell viability suggests that the extracts and fermentates derived from Savoy cabbage do not exhibit clear cytotoxic effects on the cell lines analyzed under the experimental conditions applied. Another key practical consideration involves comparing the preparations derived from Savoy cabbage leaves with those obtained from the core, which allows for an assessment of the potential for utilizing different parts of this raw material. The leaves are the primary raw material used, while the core is often treated as a by-product. Studies have shown that samples from the core do not exhibit cytotoxicity towards keratinocytes and fibroblasts, which may indicate its potential for further use as a source of bioactive ingredients. Analyses of kombucha ferments from other plant materials indicate that this process can increase the biological activity of samples while maintaining the lack of cytotoxicity to skin cells [3,48]. Nizioł-Łukaszewska et al. investigated kombucha ferments derived from white and red cabbage using HaCaT and HDF cell lines, thereby supporting the suitability of these cellular models for evaluating fermented matrices [3]. The researchers demonstrated that nanovesicles with properties similar to exosomes, derived from white and red cabbage, do not exhibit cytotoxic effects on HaCaT keratinocytes or human dermal fibroblasts (HDFs). Furthermore, it has been observed that they can stimulate the proliferation of these cells, indicating that components derived from cabbage are well tolerated by skin cells [49]. In summary, the obtained results suggest that fermented and non-fermented preparations from Savoy cabbage may be an interesting raw material for further research on ingredients with dermoprotective potential.

3.4. Assessment of Anti-Inflammatory Activity

Subsequently, the anti-inflammatory properties of extracts and fermented formulations obtained from the core, inner leaves, and outer leaves of Savoy cabbage were evaluated. Their effects were assessed by measuring the levels of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) in the human monocytic THP-1 cell line. The cells were exposed to unfermented extracts (E) as well as fermentation products obtained at 10 (F10) and 20 (F20) days, tested at concentrations of 100 and 250 µg/mL, followed by stimulation with lipopolysaccharide (LPS; 10 µg/mL) to induce an inflammatory response. Cells treated exclusively with LPS, without exposure to the extracts or ferments, were used as positive controls (PC) to represent the maximum inflammatory response. Untreated cells, receiving neither LPS nor the tested samples, served as negative controls (NC). Diclofenac (10 µg/mL) served as a reference anti-inflammatory compound, owing to its well-documented capacity to suppress the production of the inflammatory mediators examined [40]. Results for cytokine levels are presented as fold changes compared to the negative control (NC) [48]. Figure 10 illustrates that all extracts (E) and fermentates (F10 and F20) from different Savoy cabbage sections successfully decreased IL-1β production. Furthermore, LPS treatment of THP-1 cells (PC) induced a 2.69 ± 0.20-fold inflammatory elevation relative to the negative control (NC). The findings indicated that samples collected after 10 and 20 days of fermentation exhibited a more pronounced reduction in IL-1β levels than their non-fermented extract counterparts. The stronger effect was demonstrated for the outer leaves of savoy cabbage, reaching 2.28 ± 0.17-fold and 2.20 ± 0.15-fold for F10 and F20 at a concentration of 250 µg/mL, respectively.

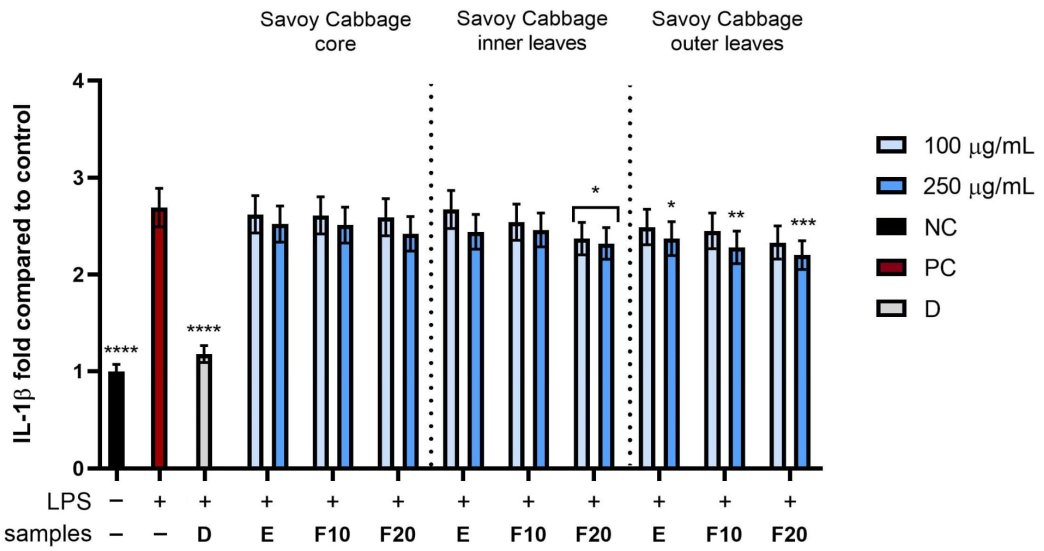


Figure 10. Effects of unfermented extracts (E) and kombucha ferments obtained after 10 (F10) and 20 (F20) days from Savoy cabbage core, inner leaves, and outer leaves on IL-1β release in LPS-stimulated THP-1. Cytokine concentrations are expressed as fold changes relative to the untreated negative control (NC). Cells treated solely with bacterial lipopolysaccharide (LPS; 10 μg/mL) served as the positive control (PC), while diclofenac (D; 10 μg/mL) was used as the reference drug. Values represent the mean ± SD derived from three independent experiments with duplicate measurements. **** $p < 0.0001$, *** $p = 0.0021$ ** $p = 0.0010$, * $p < 0.05$.

The effects of the tested extracts (E) and fermented preparations (F10 and F20) on IL-6 production were also assessed, as shown in Figure 11. The results demonstrated that, consistent with the observations for IL-1β, preparations derived from different parts of Savoy cabbage exhibited the capacity to decrease IL-6 levels. The introduction of LPS (PC) to THP-1 cell cultures initiated a strong inflammation, leading to a 3.33 ± 0.25-fold increase in the level of this cytokine compared to the control (NC). The findings indicate that fermentation markedly improved the anti-inflammatory potential of the plant material, with the fermented samples demonstrating a greater ability to reduce IL-6 levels than the corresponding conventional extracts. Notably, the cytokine suppression was time-dependent, with the 20-day fermentates (F20) showing greater inhibitory activity than the 10-day fermentates (F10). Particularly high efficiency was observed in samples obtained from the outer leaves of Savoy cabbage, where for variant F10 the result was 2.90 ± 0.22-fold, while for F20 the value was 2.66 ± 0.20-fold (for a concentration of 250 μg/mL).

The study demonstrated that both the extracts and kombucha-derived ferments obtained from different parts of Savoy cabbage displayed anti-inflammatory activity, as indicated by their ability to suppress the secretion of the pro-inflammatory cytokines IL-1β and IL-6 in LPS-stimulated THP-1 cells. However, fermentation further potentiated this biological activity, yielding maximal inhibition in the case of 20-day fermentates (F20), most notably when utilizing outer leaf tissue.

IL-1β and IL-6 are key pro-inflammatory cytokines that contribute to the immune response elicited by LPS-induced activation of monocytes and macrophages. Upon LPS stimulation, intracellular signal transduction pathways, specifically NF-κB and MAPK, are activated, driving the expression and secretion of pro-inflammatory cytokines [50,51]. Therefore, inhibition of IL-1β and IL-6 production is considered an important indicator of anti-inflammatory potential. The observed reduction in cytokine secretion after the use of both extracts and ferments suggests that bioactive compounds present in savoy cabbage disrupt these signaling pathways and attenuate the inflammatory response.

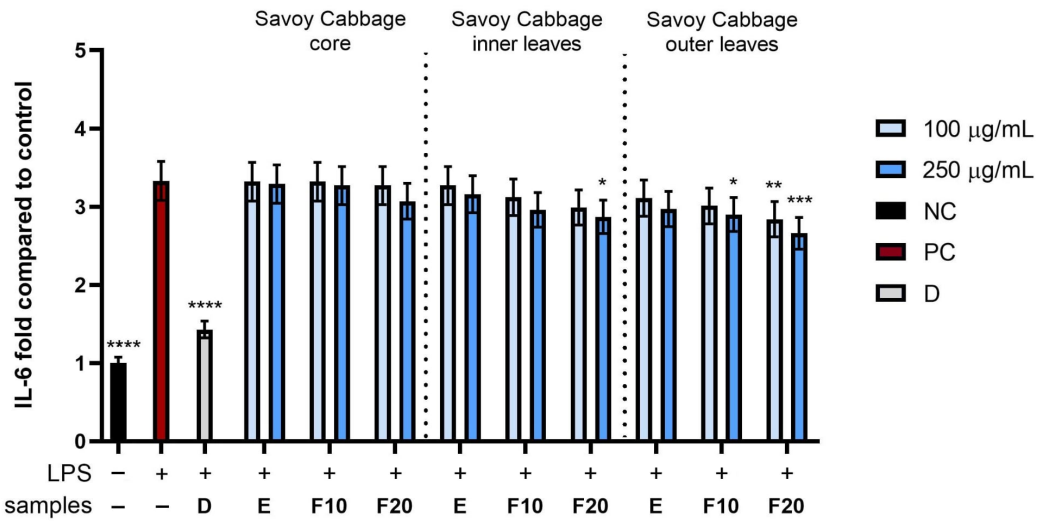


Figure 11. Effects of unfermented extracts (E) and kombucha ferments obtained after 10 (F10) and 20 (F20) days from Savoy cabbage core, inner leaves, and outer leaves on IL-6 release in LPS-stimulated THP-1. Cytokine concentrations are expressed as fold changes relative to the untreated negative control (NC). Cells treated solely with bacterial lipopolysaccharide (LPS; 10 µg/mL) served as the positive control (PC), while diclofenac (D; 10 µg/mL) was used as the reference drug. Values represent the mean ± SD derived from three independent experiments with duplicate measurements. **** $p < 0.0001$, *** $p = 0.0025$, ** $p < 0.01$, * $p < 0.05$.

The enhanced anti-inflammatory activity of kombucha ferments compared to unfermented extracts is explained by biochemical modifications driven by the fermentation process. The symbiotic culture of bacteria and yeast (SCOBY) metabolizes plant ingredients, leading to the release of lower molecular weight phenolic compounds, hydrolysis of glycosylated flavonoids and the production of organic acids and other secondary metabolites with increased biological activity [18]. These transformations often increase both the bioavailability and biological effectiveness of phytochemicals. A similar improvement in antioxidant and anti-inflammatory properties after kombucha fermentation was noted in the cases of many plant materials such as carrots, celery and parsley [20], *Tilia* L. and *Calluna vulgaris* L. flowers [52], or apple, apricot and cherry leaves [26].

The stronger activity observed for F20 compared to F10 indicates that extending fermentation to 20 days additionally promoted the formation or accumulation of anti-inflammatory metabolites. Previous studies have shown that prolonged fermentation can increase the concentration of free phenolic acids, flavonoid aglycones, glucuronic acid, gluconic acid, and other metabolites [21,53]. Chromatographic analysis performed in this study indicates the presence of, among others, chlorogenic acid and kaempferol (Table 1). Hwang et al. highlighted the pronounced anti-inflammatory efficacy of chlorogenic acid in the LPS-activated RAW 264.7 cell [54]. Supporting this, Shan et al. established that the compound downregulates cyclooxygenase-2 (COX-2) expression through the inhibition of the LPS-triggered NF-κB and JNK/AP-1 signaling routes, demonstrating its modulatory effect on inflammatory processes [55]. Nevertheless, the influence of fermentation duration is determined by the characteristics of the plant material and the microbial composition of SCOBY. Prolonged fermentation may not always be beneficial, as it can result in the breakdown of certain bioactive constituents [18,56].

Among the plant materials tested, ferments prepared from outer leaves consistently demonstrated the highest anti-inflammatory activity. Such an observation corresponds with the known localization patterns of bioactive phytochemical constituents in cruciferous vegetables [57]. Outer leaves are directly exposed to environmental stresses such as ultraviolet radiation, pathogens, and oxidative conditions, which stimulate the biosynthesis of

protective secondary metabolites. Consequently, these tissues typically contain higher concentrations of phenolic compounds, flavonoids, carotenoids, vitamin C, and glucosinolates than the inner leaves or pith. Many of these compounds exhibit potent anti-inflammatory properties by inhibiting NF-κB signaling and regulating cytokine production [57]. Fermentation can further enhance their activity by converting complex phytochemicals into more biologically active or readily digestible forms [18].

Despite the promising anti-inflammatory effects observed in this study, several limitations should be acknowledged. The investigation relied on a single in vitro model involving THP-1 monocytes stimulated with LPS. Although this experimental system is commonly used to evaluate inflammatory responses, it cannot fully reproduce the complex and multifaceted nature of inflammation under in vivo conditions. Furthermore, the specific compounds responsible for the observed biological effects were not identified. Future studies should therefore include a comprehensive phytochemical characterization of the ferments, exploration of the molecular mechanisms involving the NF-κB, MAPK, and Nrf2 signaling pathways, and validation in animal models and clinical trials.

Overall, the results show that kombucha fermentation significantly increases the anti-inflammatory potential of kale, with the greatest activity observed in ferments prepared from the outer leaves after 20 days of fermentation. These findings suggest that fermentation is an effective strategy for increasing the biological value of cruciferous vegetables and support the potential use of fermented kale as a functional food ingredient or a source of natural anti-inflammatory compounds for use in the cosmetics and pharmaceutical industries.

3.5. Assessment of Antibacterial Activity

The minimum inhibitory concentration (MIC) represents the lowest concentration of a substance required to prevent the visible growth of a microorganism. As a standard indicator of antimicrobial activity in natural matrices, this parameter allows for effective comparisons across various microbial strains [34]. Consequently, this study assessed the antibacterial effects of non-fermented extracts (E) alongside kombucha fermentates from Savoy cabbage (*Brassica oleracea* var. *sabauda* L.) leaves and core against a panel of five bacterial strains, including three Gram-positive and two Gram-negative species. The results showed that the antibacterial activity of the tested preparations differed according to the cabbage fraction used, the duration of fermentation, and the bacterial strain examined. In general, the fermented samples exhibited lower MIC values than the corresponding non-fermented extracts, indicating that kombucha fermentation enhanced the antibacterial potential of the tested material (Table 3).

Among the Gram-positive bacteria, *Staphylococcus aureus* was most effectively inhibited by preparations obtained from the outer leaves. Both 10- and 20-day ferments from this fraction showed the lowest MIC values, whereas the core extract required a markedly higher concentration to inhibit bacterial growth. A similar trend was observed for *Staphylococcus capitis*, for which fermentation clearly improved antibacterial activity, particularly in samples prepared from the outer leaves. In this case, both fermented outer-leaf preparations showed the strongest inhibitory effect. For *Micrococcus luteus*, the response was less dependent on the plant fraction, although fermentation again improved activity in most cases. The lowest MIC values were observed for the 20-day core ferment and for both fermented preparations obtained from inner and outer leaves. This suggests that, unlike the two *Staphylococcus* strains, *M. luteus* was inhibited comparably by several fermented samples rather than by one clearly dominant preparation.

The Gram-negative bacteria were generally less susceptible than the Gram-positive strains, especially *Pseudomonas aeruginosa*, which required the highest concentrations of the tested samples. Nevertheless, fermentation reduced MIC values against both *Escherichia coli*

and *P. aeruginosa*, most notably for inner- and outer-leaf preparations. In the case of *E. coli*, the strongest activity was observed for the fermented outer-leaf samples, whereas *P. aeruginosa* remained the most resistant strain, despite a clear improvement after fermentation.

Table 3. Minimum inhibitory concentrations (MIC values, µg/mL) determined for selected bacterial strains exposed to extracts prepared from the core and inner and outer leaves of Brassica oleracea var. sabauda L., including non-fermented samples (E) and preparations subjected to kombucha fermentation for 10 (F10) or 20 days (F20).

Bacteria	Minimum Inhibitory Concentration MIC [µg/mL]								
	Core			Inner Leaves			Outer Leaves		
	E	F10	F20	E	F10	F20	E	F10	F20
Staphylococcus aureus	1250	625	312	625	312	312	312	156	156
Staphylococcus capitis	625	312	312	625	312	312	312	156	156
Micrococcus luteus	625	625	312	625	312	312	625	312	312
Escherichia coli	1250	1250	625	1250	625	625	625	312	312
Pseudomonas aeruginosa	2500	1250	1250	1250	625	625	1250	625	625

Overall, the results indicate that kombucha fermentation enhanced the antibacterial properties of savoy cabbage preparations, with the most pronounced effects observed for the outer leaves. The preparations obtained through fermentation of the outer cabbage leaves exhibited the strongest antibacterial effects against *Staphylococcus* spp. and *E. coli*, whereas *P. aeruginosa* was the least responsive to the tested samples. These findings indicate that the fermentation process improves the antibacterial properties of Savoy cabbage through the synthesis or liberation of antimicrobial agents. However, the extent of this effect appears to depend on both the plant tissue used as the starting material and the bacterial species examined.

The present study demonstrated that kombucha fermentation markedly enhanced the antibacterial activity of preparations obtained from different anatomical parts of *Brassica oleracea* var. *sabauda* L. Although all fermented samples exhibited improved antimicrobial efficacy compared with the corresponding aqueous extracts, with the extent of this activity varying according to both the plant tissue type and the bacterial strain. Among the tested materials, fermented preparations obtained from the outer leaves consistently displayed the strongest antibacterial activity, whereas the crude extracts generally exhibited the weakest inhibitory effects. The results suggest that the antibacterial activity of the final preparations depends on both the initial biochemical profile of the plant material and the metabolic changes induced by the fermentation process.

One of the most interesting observations was the superior antibacterial activity of the outer leaf preparations. This finding is consistent with the physiological role of external cabbage leaves, which are continuously exposed to ultraviolet radiation, temperature fluctuations, pathogens, and herbivores. Consequently, these tissues accumulate higher concentrations of protective secondary metabolites than the inner leaves. Previous phytochemical studies on *Brassica* vegetables, including savoy cabbage, have demonstrated that the distribution of phenolic compounds differs markedly between external and internal leaves, with the former generally containing higher amounts of hydroxycinnamic acids and flavonol derivatives, particularly quercetin- and kaempferol-based compounds [38]. Such differences are believed to constitute an important component of the plant defense system and may largely explain the stronger antibacterial activity observed for fermented outer-leaf preparations in the present study.

The enhanced antibacterial activity following fermentation closely corresponded with the changes observed in the phytochemical profile. Quantitative chromatographic profiling indicated that kombucha fermentation substantially altered the phenolic composition of the analyzed material, resulting in elevated levels of a broad range of flavonoid and phenolic acid derivatives. The most pronounced changes involved kaempferol- and quercetin-based glycosides, apigenin derivatives, and catechin/epicatechin, alongside increased concentrations of gallic acid, chlorogenic acid, and galloylquinic acids. Importantly, the samples exhibiting the lowest MIC values also showed the highest concentrations of many of these compounds. Although the present study does not allow direct attribution of antibacterial activity to individual metabolites, the overall modification of the phenolic profile strongly suggests that fermentation substantially increases the concentration and/or availability of bioactive constituents responsible for bacterial growth inhibition.

These alterations in phytochemical composition align with the established metabolic functions of the microbial consortium within SCOBY. Throughout the fermentation process, yeasts and acetic acid bacteria secrete a range of hydrolytic enzymes, such as β-glucosidases and esterases, which facilitate the breakdown of complex plant tissue structures and promote the release of phenolic compounds that are initially associated with cell-wall polysaccharides. In parallel, enzymatic biotransformation may convert glycosylated flavonoids into more biologically active forms or generate new metabolites with enhanced biological activity. Therefore, the enhanced antibacterial efficacy observed after fermentation likely reflects both the increased abundance of phenolic constituents and the microbial biotransformation of these compounds into metabolites with altered chemical structures, improved bioavailability, and potentially greater antimicrobial potency [18,58].

The increased antibacterial activity was accompanied by enhanced antioxidant capacity, as demonstrated by the DPPH, ABTS and FRAP assays. Interestingly, fermented outer-leaf preparations consistently exhibited the highest antioxidant activity together with the lowest MIC values. Although antioxidant and antibacterial activities arise from different biological mechanisms, both are largely associated with the presence of phenolic compounds. This parallel increase suggests that fermentation-induced enrichment of phenolic constituents contributes simultaneously to improved radical scavenging capacity and bacterial growth inhibition. The results obtained herein are in agreement with the findings reported in the cited literature, indicating that fermentation-induced modifications of phenolic composition enhance both antioxidant capacity and antimicrobial activity in *Brassica* vegetables and other fermented plant-derived products, highlighting the central role of phenolic compounds in mediating these biological effects [3,38].

The antibacterial properties identified in this study may be explained by synergistic interactions among various groups of phenolic compounds, rather than by the activity of a single component. Phenolic acids, particularly gallic and chlorogenic acids, have been shown to compromise bacterial membrane integrity, enhance membrane permeability, disrupt cellular energy metabolism, and inhibit key enzymes essential for bacterial viability [59,60]. Flavonoids such as catechin, quercetin and kaempferol derivatives may further impair membrane integrity, disturb intracellular ATP production, alter nucleic acid synthesis and promote oxidative imbalance within bacterial cells [61,62]. Because these mechanisms target multiple cellular structures simultaneously, mixtures of phenolic compounds frequently exhibit stronger antibacterial activity than isolated constituents due to synergistic interactions. In addition to phenolic compounds, glucosinolate hydrolysis products, including isothiocyanates, may also contribute to the observed antibacterial activity. Although these compounds were not quantified in the present study, their well-documented antimicrobial properties suggest that they may act synergistically with phenolic constituents in fermented savoy cabbage preparations [63,64]. Consequently, the enhanced antimicrobial efficacy

of fermented savoy cabbage preparations is likely attributable to the collective action of fermentation-enriched phytochemicals rather than to any individual compound.

Gram-positive bacteria generally displayed greater susceptibility to the tested antimicrobial agents than Gram-negative microorganisms. The strongest inhibitory effects were observed against *Staphylococcus aureus* and *Staphylococcus capitis*, whereas *Pseudomonas aeruginosa* remained the least susceptible strain. This susceptibility pattern is consistent with numerous studies investigating the antibacterial activity of plant-derived phenolic compounds and is generally attributed to fundamental differences in the architecture and permeability of Gram-positive and Gram-negative bacterial cell envelopes. In Gram-positive bacteria, the thick peptidoglycan cell wall generally permits the diffusion of low-molecular-weight phytochemicals. Gram-negative bacteria feature an additional outer membrane enriched with lipopolysaccharides (LPS), which reduces the ability of many antimicrobial compounds to penetrate the cell and acts as a significant barrier to their entry into bacterial cells. Furthermore, species such as *P. aeruginosa* possess highly efficient multidrug efflux pumps, low outer-membrane permeability and a remarkable capacity for biofilm formation, all of which contribute to their intrinsic resistance to antimicrobial agents [65–67]. These structural and physiological characteristics likely explain the comparatively high MIC values observed for *P. aeruginosa* despite the clear improvement in activity after fermentation. Nevertheless, the marked reduction in MIC values following fermentation indicates that the fermentation-induced phytochemical modifications were sufficient to partially overcome these intrinsic resistance mechanisms, even in highly tolerant Gram-negative bacteria.

Previous studies investigating cabbage-derived preparations have likewise demonstrated greater antibacterial activity against *Staphylococcus aureus* than against Gram-negative bacteria, although the reported efficacy varies considerably depending on cabbage cultivar, extraction procedure and phytochemical composition [3]. Most published studies have focused on conventional solvent extracts or lactic acid fermentation [68], whereas quantitative MIC-based evaluation of kombucha-fermented cabbage preparations remains very limited. To the best of our knowledge, the antibacterial potential of Savoy cabbage subjected to lactic acid bacteria-mediated fermentation has received limited attention in the existing scientific literature. Most available reports concern white cabbage (sauerkraut) or kimchi fermentations and focus primarily on microbial succession, metabolite production or probiotic properties rather than direct antibacterial activity of fermented plant extracts [16,69]. Consequently, the present findings extend current knowledge by demonstrating that SCOBY-mediated fermentation enhances the antibacterial activity of different anatomical fractions of savoy cabbage and that this improvement is closely associated with fermentation-induced modifications of the phytochemical profile rather than with sample acidity alone. Since all tested preparations exhibited near-neutral pH after dilution in the assay medium, the contribution of acidity to bacterial growth inhibition is likely minimal, further supporting the role of bioactive phytochemicals and their fermentation-derived transformation products as the principal determinants of antibacterial activity.

3.6. Assessment of Transepidermal Water Loss (TEWL) and Skin Hydration

The human skin acts as a barrier organ, responsible for maintaining the body's homeostasis and protecting the body against physical, chemical and biological environmental factors. Adequate hydration of the stratum corneum is crucial for preserving the skin's proper physical properties and affects various intrinsic and extrinsic factors involved in regulating epidermal barrier function. Measuring skin hydration and transepidermal water loss is crucial for assessing the effectiveness of the epidermal barrier. Impaired epidermal barrier function can lead to excessive transepidermal water loss and reduced skin hydra-

tion. This can result in increased sensitivity to irritants, causing dry skin and potentially contributing to the development of dermatological conditions [70–73].

The research investigated the effects of extracts prepared from the core, inner leaves, and outer leaves of Savoy cabbage, alongside preparations fermented for 10 and 20 days, on important skin parameters, including hydration levels and transepidermal water loss (TEWL). The analysis was carried out at 2 h intervals following application of the preparation.

The skin hydration test demonstrated an increase in skin moisture following the application of each extract. However, the extent of hydration differed depending on the extract type and the plant part from which it was obtained. In the 2 h period following the application of the extracts under investigation, the skin hydration results can be ranked as follows: outer leaf > inner leaf > stem. For all evaluated samples, the difference in skin hydration efficacy between the unfermented extracts and the F10 and F20 ferments was statistically significant. Specifically, the values obtained for the F20 ferment derived from the core, inner leaves, and outer leaves exceeded those of their respective unfermented counterparts by 87%, 79%, and 46%. It is therefore concluded that the use of ferments instead of extracts leads to greater skin hydration. In the case of F10 ferments from the stem and inner leaves, similar results were recorded for the degree of skin hydration. By contrast, the greatest skin hydration was observed following the application of the F20 ferment from the outer leaves (Figure 12).

The study results demonstrated that TEWL decreased in relation to both fermentation duration and the time following product application. This reduction was recorded after the application of extracts derived from all the plant parts examined. However, the extent of the changes in TEWL differed among the extracts, depending on the cabbage part from which each was obtained. In the 2 h period following the application of the extracts under investigation, the TEWL results can be ranked as follows: outer leaf > inner leaf > core. For all extracts, the difference between the extract and the F10 and F20 enzymes is significant. The reduction in TEWL between the F20 enzyme and the extract is 73 percent, 68 percent and 54 percent for the stem, inner leaf and outer leaf, respectively. This indicates that using enzymes instead of the extract brings significant benefits. For the extracts (E, F10 and F20) from the stem and inner leaves, similar TEWL results were observed. If we are looking for the best result among all the samples tested, ferment F20 from the outer leaves caused the lowest loss of water from the epidermis when applied to the skin (Figure 13).

The observed patterns suggest that the phytochemical composition of cabbage extracts differs according to the plant tissue from which they are obtained. Cabbage contains various antioxidant compounds, including water-soluble vitamin C and phenolics, as well as lipid-soluble vitamin E and carotenoids, which play important roles in protecting against oxidative stress [74,75]. Furthermore, previous research [76] has established a strong relationship between the antioxidant potential of cabbage and its polyphenol concentration. Kaempferol, quercetin, and apigenin have been reported as the predominant flavonoids in cabbage. In addition, other authors [76,77] have investigated related aspects of the phytochemical composition and biological properties of cabbage. Kaempferol, quercetin and apigenin were the main flavonoids present in cabbage. Among other things, the authors of Ref. [77] found that the antioxidant capacity of cabbage was strongly correlated with its polyphenol content. Kaempferol, quercetin and apigenin were the main flavonoids present in cabbage. The authors, Zhao et al. [57], investigated the distribution profile of primary metabolites (soluble sugars, proteins, and minerals) and secondary metabolites (carotenoids, vitamin C, anthocyanins, flavonoids, and total phenolic content) across individual head cabbage leaf layers, ordered from the exterior to the core. Their findings revealed that antioxidant levels—and correspondingly, overall antioxidant capacity—decreased from the outer leaves inward. Additionally, soluble proteins and minerals followed a similar declining gradient

toward the center. In a related study, Lee et al. [78] also analyzed extracts obtained from cabbage leaves and heads in their study. They identified high levels of bioactive compounds in the cabbage leaf extract, particularly sulforaphane and vitamin C, leading to the conclusion that it holds strong potential as a natural ingredient in skin-lightening formulations. The cited studies have highlighted the considerable bioactive potential of extracts obtained from edible cabbage by-products. These extracts were reported to possess strong antioxidant, anti-inflammatory, and depigmenting properties, suggesting their applicability in the development of functional food products and cosmetic formulations. Furthermore, Jerophin et al. [79] investigated the antioxidant properties of cabbage leaf extracts incorporated into topical cream formulations. Their findings indicated that such preparations may provide effective protection against UV-induced skin damage, supporting their use in cosmeceutical applications. GC–MS profiling identified a range of potentially bioactive phytochemicals, with 19 compounds detected, including N-hexadecanoic acid, cyclohexanone, dodecanoic acid, 4H-pyran-4-one, and oleic acid. The abundance of these bioactive molecules, together with the pronounced antioxidant activity of the extracts, underscores the potential of cabbage leaf-derived preparations for cosmetic and pharmaceutical applications.

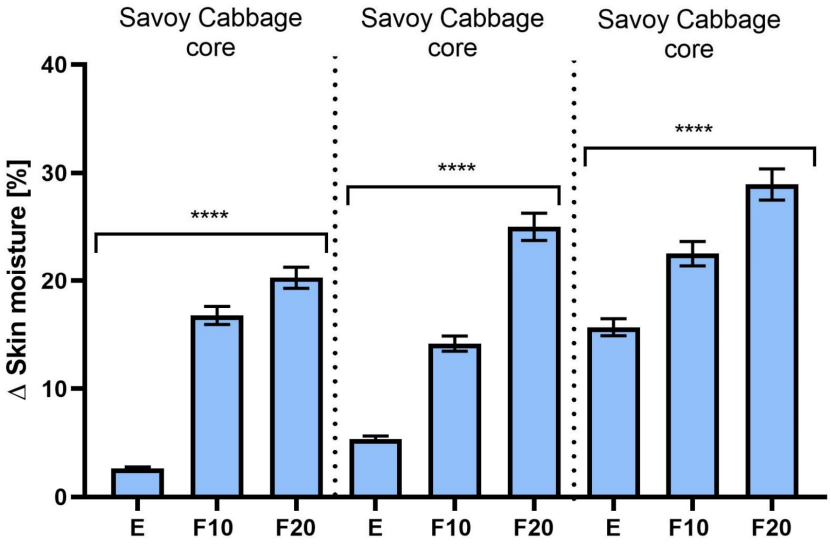


Figure 12. The influence of savoy cabbage extracts (E) and ferments (F10 and F20) on skin moisture. The results are expressed as the mean ± standard deviation (SD) from three separate experiments, each performed in triplicate. **** $p < 0.0001$.

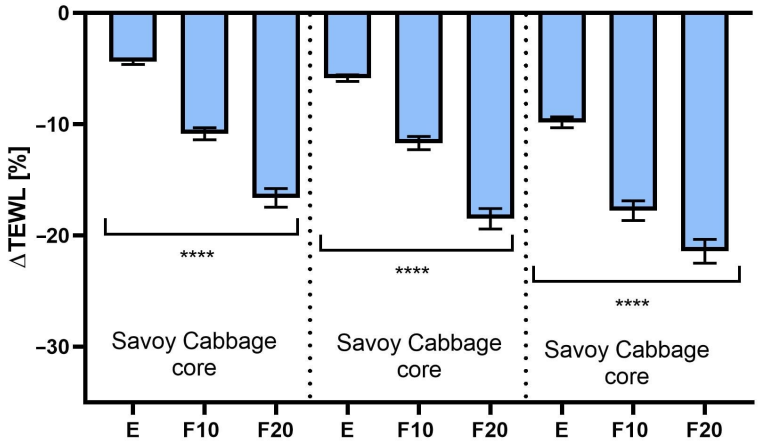


Figure 13. The influence of savoy cabbage extracts (E) and ferments (F10 and F20) on transepidermal water loss (TEWL). The results are expressed as the mean ± standard deviation (SD) from three separate experiments, each performed in triplicate. **** $p < 0.0001$.

4. Conclusions

The present work comprehensively examined the biological characteristics of preparations obtained from different morphological parts of savoy cabbage (*Brassica oleracea* var. *sabauda* L.). Both non-fermented extracts and kombucha-fermented products derived from the core, inner leaves, and outer leaves were subjected to comparative analysis. The findings revealed that kombucha-mediated fermentation significantly increased the levels of bioactive constituents relative to those detected in the corresponding non-fermented extracts. The ferments and extracts obtained from the outer cabbage leaves demonstrated stronger antioxidant, antibacterial, and anti-inflammatory properties than those produced from the inner leaves and core. Antioxidant assays further confirmed that fermented samples displayed greater free radical scavenging capacity and more effectively reduced intracellular reactive oxygen species (ROS), which was consistent with the phytochemical changes identified by the LC–MS analysis. Cytotoxicity assays revealed a concentration-dependent response, with lower concentrations maintaining or improving cell viability, whereas treatment at 500 μg/mL reduced the number of viable cells. Furthermore, kombucha-derived fermentation products exhibited a substantially greater capacity to reduce the production of the pro-inflammatory cytokines IL-1β and IL-6 compared with their corresponding non-fermented extracts. Antibacterial efficacy was influenced by fermentation time, plant tissue, and bacterial strain, with fermented preparations exhibiting progressively stronger antimicrobial activity and reaching the highest effectiveness after 10 and 20 days of fermentation. Overall, the results suggest that fermentation using SCOBY may offer an effective approach to increasing the functional potential of Savoy cabbage by-products. This process could contribute to the sustainable reuse of agricultural residues by transforming them into valuable sources of bioactive compounds for potential use in the food, nutraceutical, and cosmetic sectors. However, further studies are required to establish their safety, confirm their efficacy, and validate their suitability for practical applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app162111033/s1: Figure S1. Representative DAD chromatograms (upper panel) and the corresponding overlaid chromatograms (lower panel), recorded at $\lambda = 320$ nm, of Savoy cabbage extract (blue), the fermented extract after 20 days of fermentation (green), and the kombucha starter culture (red); Figure S2. Extracted ion chromatograms (EICs) corresponding to the characteristic m/z values of, from top to bottom: chlorogenic acid derivatives (neochlorogenic acid and chlorogenic acid), gallic acid, and p-coumaroylquinic acids isomers. Fermented extract after 20 days of fermentation (green line), and the kombucha starter culture (red line); Figure S1. Representative DAD chromatograms (upper panel) and the corresponding overlaid chromatograms (lower panel), recorded at $\lambda = 320$ nm, of Savoy cabbage extract (blue), the fermented extract after 20 days of fermentation (green), and the kombucha starter culture (red)..Figure S2. Extracted ion chromatograms (EICs) corresponding to the characteristic m/z values of, from top to bottom: chlorogenic acid derivatives (neochlorogenic acid and chlorogenic acid), gallic acid, and p-coumaroylquinic acids isomers. Fermented extract after 20 days of fermentation (green line), and the kombucha starter culture (red line)

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Abbreviations

The following abbreviations are used in this manuscript:

AB	Alamar Blue Assay
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AHA	alpha hydroxy acids
CFU	colony forming units
CO_2	carbon dioxide
COX-2	cyclooxygenase 2
DAD	diode array detector
DCF	2′,7′-dichlorofluorescein
DMEM	Dulbecco's modified eagle medium
DPPH	1,1-diphenyl-2-picrylhydrazyl
E	extract
ELISA	enzyme-linked immunosorbent assay
ESI/TOF	Electrospray Ionization/Time-of-Flight
F10	10-day ferment
F20	20-day ferment
FBS	fetal bovine serum
FRAP	ferric reducing antioxidant power
H_2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
H_2O_2	hydrogen peroxide
HaCaT	human keratinocyte cell line
HDF	human dermal fibroblasts
IL-10	interleukin 10
IL-1β	interleukin-1 beta
IL-6	interleukin 6
iNOS	inducible nitric oxide synthase
LPS	lipopolysaccharide
MIC	minimum inhibitory concentration
NF-κB	nuclear factor kappa B
NO	nitric oxide
NR	Neutral Red Assay
PBS	phosphate-buffered saline
RAW264.7	murine macrophage cell line
ROS	reactive oxygen species
SCOBY	symbiotic culture of bacteria and yeast
TEWL	transepidermal water loss
THP-1	human acute monocytic leukemia cell line
TNF-α	tumor necrosis factor alpha
TPTZ	2,4,6-Tripyridyl-s-triazine
UHPLC	ultra-high-performance liquid chromatography
UV-VIS	ultraviolet-visible spectroscopy

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