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Potential Application of a Wine Extract in Skin Care: How to Benefit from the Antibacterial, Antioxidant and Elastase Inhibiting Properties

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Abstract

Since plant polyphenols have many beneficial properties on health, the aim of this study was to evaluate the potential use of a phenolic wine extract, a by-product of wine production, for skin care on HaCaT cells. In these studies, a significant reduction of reactive oxygen species formation in HaCaT cells and severe elastase inhibition was observed. In contrast, the wine extract caused a major increase in lipase activity. The extract showed no influence on cell proliferation, but an immunomodulatory effect on the release of the interleukins IL-6 and IL-8 was found. The phenolic wine extract demonstrated a strong activity against gram-positive and gram-negative pathogens, yeasts, and fungi. Overall, our results show that the investigated phenolic wine extract is a promising ingredient for anti-aging skin care, could contribute to the improvement of skin appearance and health, and may positively affect cellulite.

Keywords

Phenolic Wine Extract, Skin Care, HaCaT Cells, Elastase, Lipase, IL-6, IL-8, Cell Proliferation, Reactive Oxygen Species, Antimicrobial, Antimycotic

1. Introduction

The skin, the largest organ of the human body, fulfills a variety of active functions that predominantly protect the body from potential risks but also regulate physiological processes and transmit sensory impressions [1]. It protects the organism from mechanical, chemical, physical, and microbial influences and is in constant interaction with the entire organism [2]. The slightly acidic hydrolipidic film of the skin, with a pH value of approximately 5.5, provides ideal condi-

tions for the physiological resident skin flora and thus prevents colonization with pathogenic microorganisms [3]. The skin is composed of three layers, with the outermost layer, the epidermis, consisting mainly of keratinocytes and representing the organism's first immune barrier [4]. The keratinocytes located in the epidermis are capable of producing many different cytokines, growth factors, and complement factors, and are significantly involved in the wound healing process [5]. Moreover, the human skin also has an enormously valuable psychological function, as it significantly influences and defines our external appearance. With age, the changes in our skin become noticeable mainly through loss of elasticity, appearance of age spots, wrinkles, dyspigmentation, and dysfunction of wound healing and barrier function [6] [7] [8]. The most important alterations in aging skin occur at the level of dermal connective tissue, and are characterized by the loss of mature collagen and changes in the elastic network [9]. The degradative enzymes of this network include matrix metalloproteinases and elastases, which control important processes, such as wound healing and cell migration through the turnover of various extracellular matrix proteins [10]. However, excessive levels of these enzymes are a major cause of various skin disorders, including rapid skin aging [11].

With skin aging, a fundamental distinction needs to be made between intrinsic, natural, and genetically caused skin aging and extrinsic skin aging [12]. Extrinsic skin aging is controlled by contact with the environment and its pollutants, one of the major influencing factors being UV radiation [13]. Both types of aging differ notably in terms of phenotype [14], where extrinsic skin aging causes severe visible damage. In particular, extrinsic skin aging induces the formation of reactive oxygen species (ROS) [15] and degradation of endogenous antioxidants [16], which may lead to enormous cellular damage and additionally affect the activity of matrix metalloproteinases [17]. This results in enhanced tissue degradation and slower regeneration.

As our most exposed organ, confronted with various external stimuli, it is necessary to protect our skin and its integrity, as well as its barrier function. In addition, it is desirable to keep the outer appearance as impeccable as possible and to counteract respectively prevent extrinsic skin aging by appropriate care.

Every year, tons of grapes are pressed for wine production worldwide, leaving behind a large amount of phenol-rich byproducts such as seeds, grape skins, and stems [18] [19]. These by-products require a complex disposal process, as the contained polyphenols can destroy the sensitive balance of the ecosystem [20]. Since Polyphenols are considered to have health-promoting, anti-inflammatory, antimicrobial, and antioxidant properties, the by-products of wine production have great potential for use in skin care due to their high polyphenol content [21].

In this work, a phenolic wine extract was investigated regarding its potential for skin care. Various *in vitro* assays were performed on HaCaT cells to evaluate its impact on inflammatory processes, pathogenic skin organisms, and potential

protective properties with respect to skin aging. Additionally, the effect of the extract on the enzyme lipase was investigated.

2. Materials and Methods

2.1. Wine Extract and Sample Preparation

The wine extract used in this study was provided by Fattoria La Vialla (Arezzo, Italy) and the composition of the batch was provided by a certificate of analysis (Table 1). Phenol-rich wine extract is a by-product of wine production and contains 13% ethanol. To obtain the extract, the separation of the stems and pressing of the grapes were followed by a twelve-day maceration at 26˚C. The liquid supernatant was then decanted for wine production. The residue (skin

Table 1. Quantification of the ingredients in the wine extract according to the certificate of analysis provided by the manufacturer Fattoria La Vialla.

Ingredient	Concentration [mg/L]
Caffeic acid	3.344
Coumaric acid	0.931
Ferulic acid	0.477
Gallic acid	29.557
Caffeoyl tartaric acid	67.953
Cumaryl tartaric acid	14.003
Ferulitartaric acid	8.791
Quercetin-3-O-glucoside	6.033
Quercetin-3-O-galattoside	1.979
Quercetin-3-O-glucuronide	15.240
Quercetin-3-O-rutinoside	<0.050
Isorhamnetin	0.153
Kaempferol	0.067
Myricetin	0.086
Quercetin	10.933
trans-Resveratrol	24.202
(−) Epicatechin	28.120
(+) Catechin	92.035
Procyanidin B1	42.673
Procyanidin B2	33.947

and seeds) called wine pomace, plus a small amount of remaining liquid was squeezed, and the obtained liquid was filtered (0.2 µm, ceramic membrane). This filtrate, a phenol-rich wine extract, was used in the following studies. Prior to use, the extract was sonicated for 10 min, centrifuged at 500 x g for 20 s, and filtrated (0.2 µm). For all *in vitro* assays, final dilutions were prepared with cell growth medium for HaCaT cells (Dulbecco's modified Eagle medium with stable glutamine and sodium pyruvate with a glucose concentration of 1 g/L supplemented with 10% Fetal Bovine Serum, Capricorn Scientific, Ebsdorfergrund, Germany).

2.2. Cultivation of HaCaT-Cells

Human adult low calcium high temperature keratinocytes (HaCaT) obtained from AddexBio (San Diego, CA, USA) were cultured at 37˚C and 8.5% CO_2 in Dulbecco's modified Eagle medium with stable glutamine and sodium pyruvate (Capricorn Scientific, Ebsdorfergrund, Germany) with a glucose concentration of 1 g/L supplemented with 10% Fetal Bovine Serum (Capricorn Scientific, Ebsdorfergrund, Germany).

2.3. Cell Viability Assay

The effect of the wine extract on the viability of HaCaT cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay in accordance with Mosmann [22]. HaCaT cells were seeded in 96-well plates (40,000 cells per well) and incubated overnight at 37˚C and 8.5% CO_2. Cells, were then washed with phosphate-buffered saline (PBS), and the samples were applied for 24 h (100 µL) respectively 48 h (200 µL). After sample incubation, 0.25 mg/mL MTT (VWR International, Radnor, PA, USA) in cell growth medium was added, and the plate was incubated at 37˚C for 1 hour. Subsequently, the MTT solution was removed, and cells were lysed with dimethyl sulfoxide (Merck KgaA, Darmstadt, Germany) while shaking at 300 rpm for 1 hour. Finally, the absorbance was measured at 570 nm and 650 nm (reference wavelength) using Synergy™ HTX multi-mode microplate reader with the software Gen5™ 2.07 (BioTek Instruments Inc., Winooski, VT, USA). Results are shown in relation to the untreated control (incubated with cell culture medium or incubated with 1.3% ethanol in medium), and dimethyl sulfoxide served as blank value. All experiments were performed in three independent runs with six replicates.

2.4. Cell Proliferation Assay

To study the effect of wine extract on cell proliferation, 20,000 HaCaT cells per well were seeded in a 96-well plate, and after 24 h incubation at 37˚C and 8.5% CO_2, the cultivation medium containing only 0.05% Fetal Bovine Serum was applied. After a further 24-hour incubation period, the different dilutions of the wine extract (prepared with serum-free growth medium) were applied. As controls, cells were treated with 0.02 ng/mL epidermal growth factor in serum-free

growth medium (positive control) and with serum-free medium only (negative control). Cells were incubated with the corresponding samples for 24 h (100 µL) respectively 48 h (200 µL) at 37˚C and 8.5% CO_2. At the end of incubation, MTT assay was performed in accordance to the cell viability assay. Subsequently, cell viability was calculated in relation to the untreated control (incubation with serum-free medium). The experiments were performed in three independent runs, each with six replicates.

2.5. Anti-Inflammatory Assay *in Vitro*

HaCaT cells were cultured in 24-well plates (20,000 cells per well) in 1 mL growth medium for 24 h at 37˚C and 8.5% CO_2. Cells were then washed with PBS, and the samples were applied for 24 h (0.5 mL), and 48 h (1 mL). The inflammatory response was induced by the co-application of 10 ng/mL TNF-α (R&D Systems, Wiesbaden, Germany), and the anti-inflammatory drug hydrocortisone (10^{-6} M, Sigma Aldrich, St. Louis, MO, USA) was used as the positive control. At the end of incubation, cell-free supernatants were collected, and the concentrations of Interleukin-6 (IL-6, Thermo Fisher Scientific, Waltham, MA, USA) and Interleukin-8 (IL-8, R&D Systems Inc. Minneapolis, MN, USA) were determined using commercial ELISA kits according to the manufacturer's protocols without adaptions. The absorbance was measured at 450 nm and 570 nm (reference wavelength) using Synergy™ HTX multi-mode microplate reader with the software Gen5™ 2.07 (BioTek Instruments Inc., Winooski, VT, USA), and the results were calculated using four-parameter logistic regression in GraphPad Prism® v8.3.0 (538) (San Diego, CA, USA). Results are shown in relation to the untreated control. All experiments were performed in three independent runs with three replicates.

2.6. Reactive Oxygen Species Assay *in Vitro*

To determine the antioxidative activity of the extract 40,000 HaCaT cells per well were seeded in a black 96-well plate with a clear bottom (Corning Life Science, Tewksbury, MA, USA) and incubated overnight at 37˚C and 8.5% CO_2. In this particular experiment, a growth medium without phenol red was used (Capricorn Scientific, Ebsdorfergrund, Germany). Cells were washed twice with PBS, and 100 µL of the samples containing 50 µM 2,7-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA, Sigma-Aldrich, St. Louis, MO, USA) were applied for 30 min (37˚C). The application of 100 µL H_2O_2 (100 µM; Sigma-Aldrich, St. Louis, MO, USA) for 45 min (37˚C and 8.5% CO_2) was done after another washing step with PBS. A final washing step with PBS was carried out before measuring the fluorescence intensity (FLU, ex./em. 485/528 nm) with the Synergy™ HTX (BioTek Instruments Inc., Winooski, VT, USA) using the following measuring parameters 100 µL of phosphate buffer, area scan with 5x5 measuring points, bottom read. The $DCFH_2$ radical scavenging activity was calculated based on Equation (1). All experiments were performed in three independent runs with

three replicates.

$$\text{DCFH}_2 \text{ radical scavenging activity } (\%) = 100 \times \frac{\text{FLU}_{\text{control}} - \text{FLU}_{\text{sample}}}{\text{FLU}_{\text{control}}} \quad (1)$$

2.7. Assessment of the Antimicrobial and Antimycotic Properties

The antimicrobial and antimycotic properties of the extract were evaluated in a suspension test according to DIN EN ISO 11930:2019-04 [23]. In the test procedure, the effect on the pathogens *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus brasiliensis* was investigated. For this method, the undiluted extract was inoculated with the individual microorganisms, a bacterial concentration between 10^5 and 10^6 CFU/g and a mold concentration between 10^4 and 10^5 CFU/g. These samples were incubated at 22.5˚C ± 2.5˚C, and growth control was performed immediately after inoculation, *i.e.*, after one, two, and three weeks of incubation by determining the colony-forming units (CFU). CFU were used to calculate the logarithmic reduction rate (log10) for the respective pathogen. The method was performed in three independent experimental runs.

2.8. Elastase Inhibition Assay

The elastase inhibitory activity was determined following Bieth *et al.* [24], whereby the assay was adapted to a 96-well plate format. Into each Well 120 µL of 100 mM Tris-HCl buffer (pH 8.0), 20 µL of 0.175 U/mL elastase (Sigma Aldrich, St. Louis, MO, USA), and 20 µL of the sample were added. For the negative control, the sample volume was supplemented with buffer, and for the positive control, 20 µL of 5.85 µg/mL elastinal (Merck Millipore, Burlington, MA, USA) replaced the sample. After incubation for 30 min at 25˚C and 300 rpm (orbital shaker), 40 µL of 8.75 mM N-succinyl-Ala-Ala-p-nitroanilide was added, and absorbance at 410 nm for 30 min (20 s intervals, 25˚C) was measured (Synergy™ HTX multi-mode microplate reader with the software Gen5™ 2.07, BioTek Instruments Inc., Winooski, VT, USA). 140 µL Tris-HCl buffer with 20 µL sample, and 40 µL substrate served as blank. The relative inhibition activity was determined according to Equation (2).

$$\text{elastase inhibition}(\%) = 100 \times \frac{\text{slope}_{\text{negativecontrol}} - \text{slope}_{\text{sample}}}{\text{slope}_{\text{negativecontrol}}} \quad (2)$$

After subtracting the blank value, the slope (up to 5 min after the addition of the substrate) was determined in the linear range of the curve. Based on these results, the half maximal inhibitory concentration (IC_{50}) was calculated using GraphPad Prism® v8.3.0 (538) (San Diego, CA, USA). The experiment was performed three times with three replicates.

2.8. Lipase Activity Assay

The lipase activity was quantified by a colorimetric enzyme assay in 96-well plate

format. Into each well 120 µL of 200 mM Tris-HCl buffer (pH 8.5), 20 µL of 1 mg/mL lipase (Sigma Aldrich, St. Louis, MO, USA) and 20 µL of sample were added. For the negative control, the sample volume was supplemented with buffer, and for positive control 20 µL of 0.5 µg/mL tetrahydrolipstatin (Santa Cruz Biotechnology, Dallas, TX, USA) replaced the sample. After incubation for 30 min at 37˚C and 300 rpm (orbital shaker), 40 µL of 6.5 mM 4-nitrophenyl octanoate was added and absorbance was measured at 410 nm for 60 min (20 s intervals, 37˚C) using the Synergy™ HTX multi-mode microplate reader with the software Gen5™ 2.07 (BioTek Instruments Inc., Winooski, VT, USA). 140 µL Tris-HCl buffer, 20 µL sample and 40 µL substrate served as blank. The relative lipase activity was determined according to Equation (3).

$$\text{lipase activity}(\%) = 100 \times \frac{\text{slope}_{\text{sample}}}{\text{slope}_{\text{negativecontrol}}} \tag{3}$$

After subtracting the blank value, the slope of the curve was calculated at time 26.55 min. The experiment was performed three times with three replicates.

2.9. Statistical Analysis

Data are given as arithmetic mean values ± standard deviation. To verify statistically significant differences between the mean values of different groups, a two-way ANOVA followed by a Dunnett test was carried out. Thus, $p \leq 0.05$ is considered to indicate a significant difference. Statistical analysis was performed using GraphPad Prism® v8.3.0 (538) (San Diego, CA, USA).

3. Results

3.1. Wine Extract Modulates the Immune Response of HaCaT Cells after Stimulation with TNF-α

The influence of the wine extract in different dilutions on HaCaT cell viability was examined by MTT assay over 24 h and 48 h to identify which dilutions are suitable for the following *in vitro* experiments. In relation to the untreated control, cells treated with the wine extract exhibited viabilities ranging from 67.60% ± 3.93% (1:10 dilution, 24 h) to 112.72% ± 6.05% (1:500 dilution, 48 h). Based on the results (**Figure 1**), the dilutions 1:100 and 1:200 were selected for immunomodulation studies.

An initial increase in IL-6 and IL-8 release after 24 hours with simultaneous application of TNF-α and the samples was followed by a decrease in IL release at 48 hours at both concentrations (**Figure 2**). Compared to the untreated control, IL-6 release was even reduced by 14.27% ± 5.44% (1:200 dilution) after 48 hours. Interleukine-8 was secreted to a higher extent when incubation with both samples (1:100: 34.09% ± 5.90% 24 h, 20.47% ± 8.91% 48 h, 1:200: 29.23% ± 16.37% 24 h, 19.75% ± 15.46% 48 h). As expected, the anti-inflammatory drug hydrocortisone (positive control) resulted in decreased release of both interleukins.

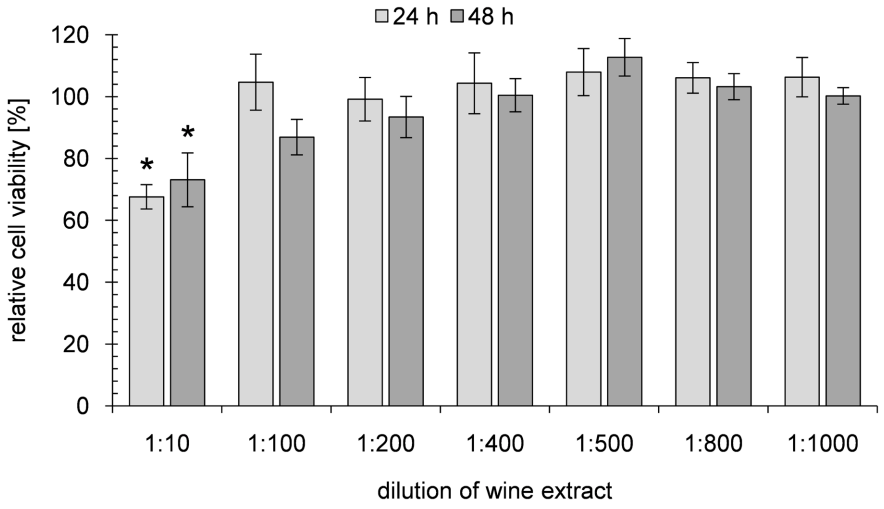

Figure 1. Viability of HaCaT cells after 24 h and 48 h incubation with wine extract using MTT assay with 40,000 cells/well. Viability was calculated in relation to the untreated control. Mean ± standard deviation of three independent experiments with six replicates each, *$p \leq 0.05$.

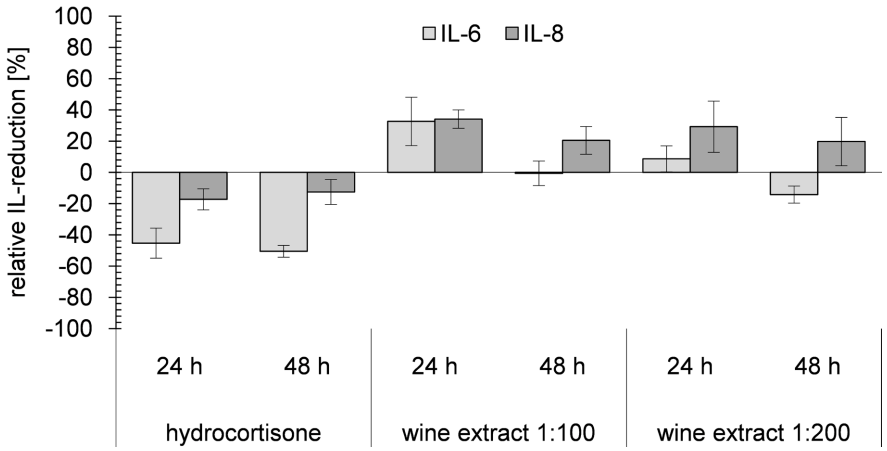

Figure 2. Relative IL-6 and IL-8 release in HaCaT cells stimulated with TNF-α (10 ng/mL) after 24- and 48-hours incubation. Wine extract at dilutions of 1:100 and 1:200 were applied at the same time. Hydrocortisone (10^{-6} M) served as a positive control for an immunomodulatory effect. IL reduction was calculated relative to cells treated with TNF-α only. Mean value ± standard error of mean of three independent experiments with three replicates each.

3.2. Wine Extract Does Not Affect the Proliferation of HaCaT Cells

Studies on the proliferation of HaCaT cells showed no enhancing effect of the wine extract after 24 and 48 hours, respectively (**Figure 3**). Cell viability ranges from 83% to 100% after treatment with the wine extract in serum-free medium. In comparison, the epidermal growth factor (EGF), positive control, showed a time-dependent and significant increase in cell viability and, consequently, a proliferation-promoting effect. Cell viability of HaCaT cells was 145.51% ± 8.51% (24 h) and 208.96% ± 13.43% (48 h), respectively, after treatment with EGF compared to the untreated control.

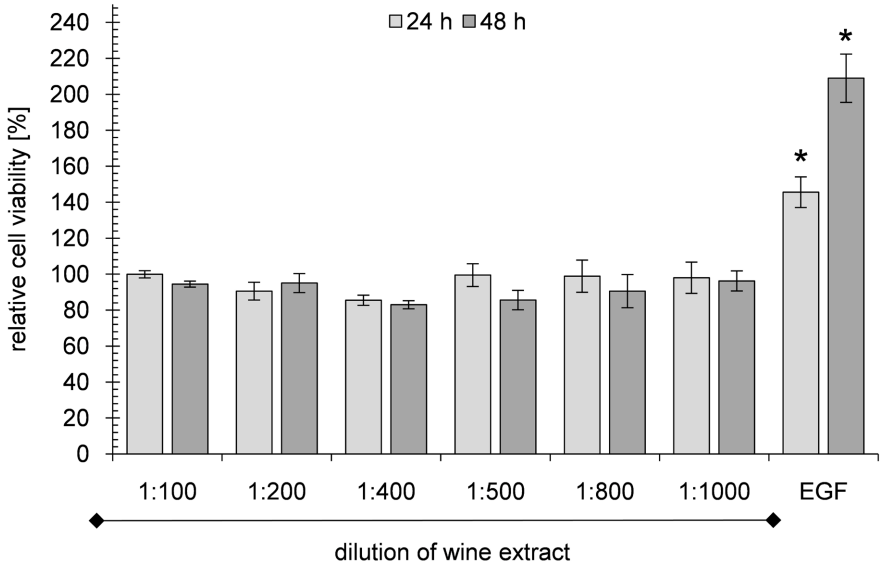

Figure 3. Proliferation of HaCaT cells after 24- and 48-hours incubation with the wine extract in serum-free medium using MTT-assay. Viability was calculated relative to cells treated with serum-free medium only (untreated control). 0.02 ng/mL EGF diluted in serum-free cell culture medium served as positive control. Mean ± standard deviation of three independent experiments with eight replicates each, *$p \leq 0.05$.

3.3. Wine Extract Inhibits the Growth of Selected Pathogenic Skin Organisms

To evaluate the antimicrobial potential of the wine extract, the antifungal and antibacterial properties were investigated with regard to various pathogenic organisms. In this case, it was shown that the colony-forming units of the bacteria *E. coli*, *S. aureus*, *S. epidermidis*, *P. acnes*, and *P. aeruginosa* were reduced by 99.99% after an incubation period of 7 days (**Table 2**). After 28 days, a 99.9% reduction was observed in all organisms tested.

Table 2. Reduction of viable organisms caused by the wine extract after 7 d, 14 d, and 28 d of incubation. The experimental set-up followed the suspension method by inoculating the undiluted extract with the specified microorganisms (colony-forming units, CFU/g).

Microorganism	CFU/g	Reduction [%]		
		7 d	14 d	28 d
A. brasiliensis	4.6×10^4	<90.0	>99.0	>99.9
C. albicans	3.0×10^4	>99.9	>99.9	>99.9
E. coli	1.7×10^5	>99.99	>99.99	>99.99
S. aureus	1.7×10^5	>99.99	>99.99	>99.99
S. epidermidis	5.0×10^5	>99.99	>99.99	>99.99
P. acnes	1.2×10^5	>99.99	>99.99	>99.99
P. aeruginosa	1.7×10^5	>99.99	>99.99	>99.99

3.4. Wine Extract Reduces Formation of Reactive Oxygen Species and Inhibits the Tissue-Degrading Enzyme Elastase

To investigate the effect of the wine extract on the formation of reactive oxygen species in HaCaT cells, an *in vitro* assay based on the intracellular transformation of the $DCFH_2$-DA dye was used. The formation of ROS was induced by treating the cells with H_2O_2, and ascorbic acid (100 µM) served as a positive control. The wine extract was applied in the dilution 1:10, 1:20, and 1:40 in growth medium without phenol red. The wine extract reduces intracellular ROS formation in a concentration-dependent manner, and the 1:10 dilution leads to a stronger reduction of ROS compared to ascorbic acid (**Figure 4**). The wine extract led to a reduction of free radicals by 49.35% ± 5.21% (1:10), 12.13% ± 4.39% (1:20), and 9.30% ± 2.82% (1:40), respectively.

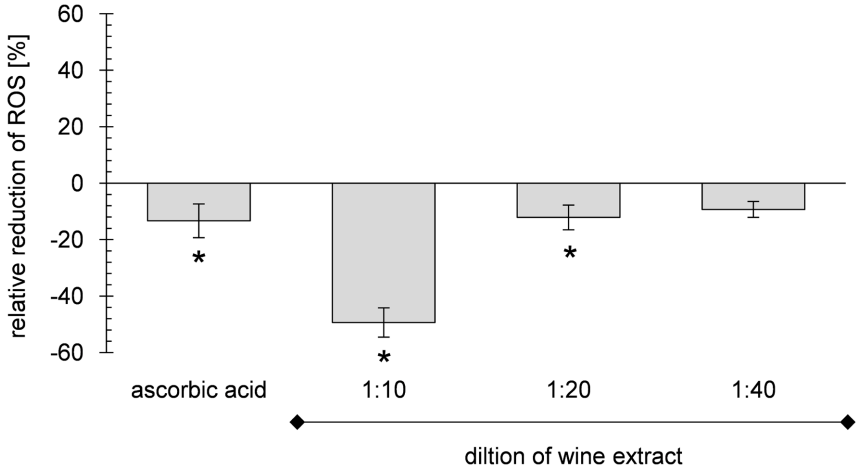

Figure 4. Relative ROS-reduction by pretreatment (30 min) of HaCaT cells with different samples (wine extract and ascorbic acid (100 µM), respectively) to the untreated control 100%. ROS formation was induced by 45 min treatment with H_2O_2. Mean ± standard deviation of three independent runs with eight repetitions * $p \leq 0.05$.

The studies on the potential of the extract to inhibit the tissue-degrading enzyme elastase showed a concentration-dependent effect, too. Different dilutions of the wine extract led to an overall inhibition of elastase of up to 78.31% ± 2.51% (**Figure 5**). Further dilutions demonstrate inhibition of 62.74% ± 5.91% (5% extract), 45.34% ± 2.04% (2.5% extract), 23.33% ± 7.02% (0.625% extract), 19.64% ± 1.46% (0.3125% extract) and 16.84% ± 2.12% (0.15625% extract). The mean inhibitory concentration (IC_{50}) with respect to the enzyme elastase was found at a wine extract concentration of 3.87% ± 1.37%. The included inhibitor elastinal indicates an inhibition of elastase of 57.22% ± 0.60%.

3.5. Wine Extract Influences Lipase Activity

Studies on the modulation of the lipase enzyme activity showed an increase in enzymatic activity after incubation with the wine extract (**Figure 6**). The wine extract enhances the conversion of lipase up to 69.18% ± 6.57% (10% extract).

This concentration-dependent effect decreases up to a lipase activity of 114.40% ± 1.09% with a concentration of 0.15628% wine extract. The included inhibitor orlistat showed the expected inhibition of lipase.

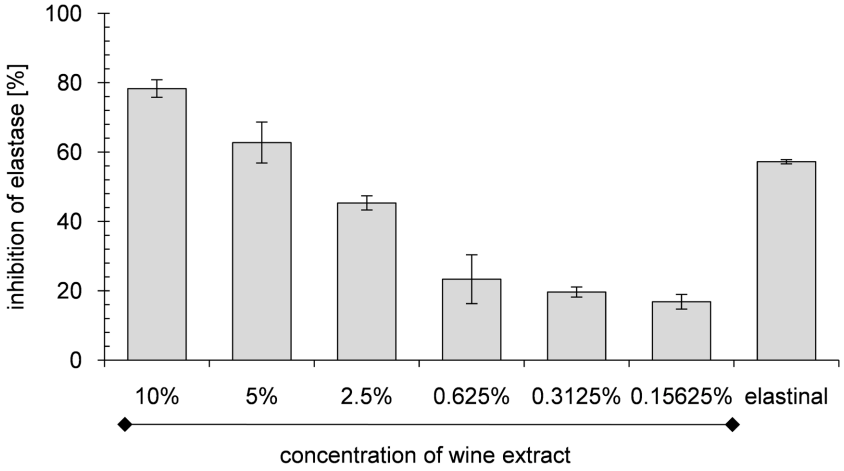

Figure 5. Inhibition of the enzyme elastase by different wine extract concentrations relative to the untreated control. The inhibitory potential was determined in a colorimetric enzyme assay at 25˚C. The elastase inhibitor elastinal (5.85 µg/mL) served as a positive control. Mean ± standard deviation of three independent experiments with three replicates each.

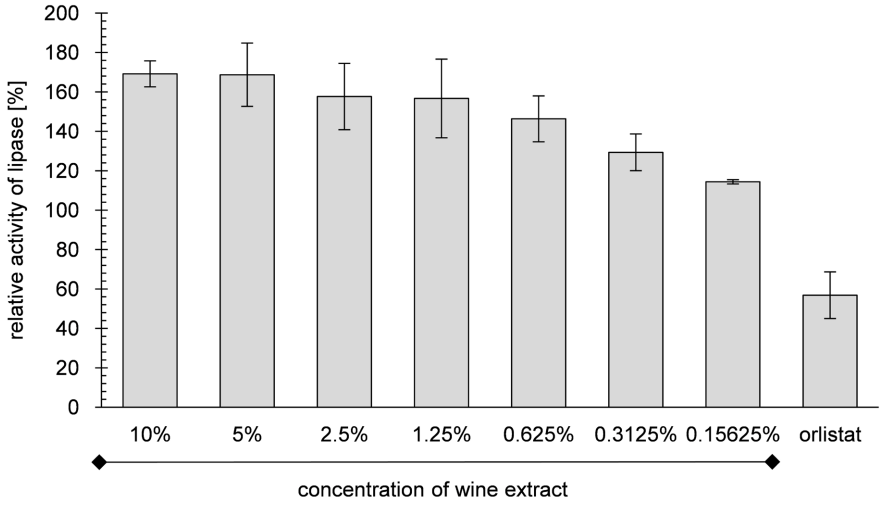

Figure 6. Activity of the enzyme lipase after incubation with different wine extract concentrations relative to the untreated control. Determined in a colorimetric enzyme assay at 37˚C. The lipase inhibitor orlistat served as a positive control. Mean ± standard deviation of three independent experiments with three replicates each.

4. Discussion

In the present work, the potential of a wine extract with a high content of polyphenols was investigated. Polyphenols occur in plants, especially in the peels of fruits and leaves, as metabolic products of secondary metabolism and contribute to their taste or appearance, e.g. as colorants, fragrances, or aromatic substances

[25]. Since wine polyphenols have numerous beneficial properties and can act antimicrobial [26] [27], anti-inflammatory [28] [29], and antioxidant [30], the potential of this wine extract for skin care was investigated. The main components of the evaluated wine extract are catechin, caffeoyl tartaric acid, procyanidin, gallic acid, epicatechin, resveratrol, and quercetins (**Table 1**). These ingredients have also been identified in other pomace, seed, or skin-based wine extracts [31] [32] [33].

To examine a possible immunomodulatory effect of the extract, the release of the interleukins IL-6 and IL-8 was studied after stimulation with TNF-α at two time points. Both cytokines play a significant role in various inflammatory skin diseases such as psoriasis [34] or atopic dermatitis [35] and are pro-inflammatory interleukins that are additionally involved in wound healing [36] [37]. The wine extract increased the release of IL-8 over the period of study, whereas IL-6 release was reduced after 48 h (**Figure 2**). The observed decrease in IL-6 release may be attributed to the containing resveratrol. Wang *et al.* [38] previously demonstrated that resveratrol reduces the secretion of interleukin-6 in HaCaT cells pre- and post-transcriptionally. *In vivo*, the application of this substance also significantly reduced IL-6 release in severe burns [39]. Additionally, several studies have reported that catechin [40] and procyanidin [41], both components of the investigated wine extract, can reduce pro-inflammatory cytokines. The glucocorticoid hydrocortisone, an anti-inflammatory drug, reduced the release of IL-6 and IL-8 in HaCaT cells in our experiments, which is in accordance with other studies [42] [43].

The demonstrated, stimulating effect of the positive control EGF (**Figure 3**) on the proliferation of HaCaT cells is similar to the results of Liang *et al.* [44]. In their studies, higher EGF concentration (20 ng/mL) also led to increased HaCaT proliferation of approximately 200% after 48 h. No effect on cell proliferation was observed with the investigated wine extract. The contained phenolic acids, such as gallic acid, have already shown anti-proliferative effects *in vitro*, especially on cancer cells [45]. Similar effects are reported about resveratrol [46], a reduced proliferation of cancer cells, and inhibition of the proliferation of human keratinocytes. Anti-proliferative properties of various wine extracts have already been demonstrated *in vitro* with different cell types [47] [48].

Screening of the antimicrobial and antimycotic properties of the wine extract revealed a strong reduction in the colony-forming units of microorganisms. The wine extract was effective against gram-positive and gram-negative bacteria, yeasts, and fungi (**Table 2**). These profound antimicrobial and antimycotic effects are caused by the ingredients catechin [49], resveratrol [50], quercetin [51] and caffeoyl tartaric acid [52]. Due to these properties, the wine extract could inhibit or prevent the growth and colonization of pathogenic microorganisms on the skin. Since these pathogens are frequently associated with inflammatory skin diseases [53] and chronic wound infections [54], the use of wine extract may have a positive effect on microbiome balance.

To address the possible application of the wine extract in anti-aging products, two important components of skin aging, formation of reactive oxygen species and influence on the enzyme elastase, were investigated. Skin cells are permanently exposed to reactive oxygen species generated either by cell metabolism [55] or by external stimuli such as UV radiation [56]. If insufficient antioxidant substances are present in cells, excess reactive oxygen species due to oxidative stress [57] can activate signaling pathways of skin aging, trigger inflammation, or lead to apoptosis [58]. The investigated wine extract reduced ROS formation in a concentration-dependent manner (**Figure 4**). In fact, less ROS were produced compared to the well-known antioxidant ascorbic acid. This observation is particularly due to the substances resveratrol [59], procyanidin [60], and quercetin [61]. Studies by Perra *et al.* [32] and Maluf *et al.* [62] also demonstrated the antioxidant activity of various wine extracts in a fibroblast mouse cell line (3T3), in which similar ingredients were found.

In addition to ROS, the enzyme elastase is a major contributor to skin aging. This enzyme is responsible for the cleavage of elastin, an important protein in the extracellular matrix, that is essential for the firmness and elasticity of the skin [63]. We observed that our wine extract inhibited the activity of elastase in a concentration-dependent manner up to approximately 80% (**Figure 5**); this was confirmed by other studies on wine extracts [64] [65]. These inhibitory properties can probably be attributed to the presence of quercetin and gallic acid. Moreira *et al.* [66] demonstrated in an elastase inhibition assay that the mixture of these two substances and ellagic acid leads to a concentration-dependent inhibition of elastase. These results, as well as the data obtained in our experiments, highlight the potential of our wine extract in terms of its use in anti-aging skin care.

Moreover, the influence of the wine extract on the enzyme lipase was investigated, too. A concentration-dependent increase in lipase activity was observed (**Figure 6**). Lipase is an important lipolytic enzyme that cleaves intracellular lipids into fatty acids and glycerol, resulting in free fatty acids that can be excreted by the cells [67]. Due to the strong increase of lipase activity by the wine extract, a positive effect on cellulite reduction is possible. Cellulite can be caused by various factors, but it has been found that inflammation of connective tissue is often present and there is an imbalance between lipolysis and lipogenesis in the adipocytes [68]. Consequently, the amount of triglycerides stored in adipocytes increases, causing them to become larger. By activating intracellular lipolysis, triglycerides could be discharged from the cells again, causing them to reduce in size and thus have a positive effect on cellulite.

This work has shown that the wine extract as a by-product of wine production has numerous positive properties that could be used, particularly in skin care, without time-consuming and cost-intensive processing steps, thus representing a very sustainable resource. Nevertheless, the next step should be to develop a suitable cosmetic formulation for dermal application and to verify the investiga-

tions by *in vivo* studies. It is important to note that the wine extract is a mixture of a variety of ingredients whose individual effects have not yet been fully elucidated to date. Therefore, it would be of particular interest to further analyze the mixture with regard to the individual components and their effects in future studies. However, synergistic effects cannot be ruled out either. For the subsequent use of the plant extract, the influences of the cultivation and the processing procedure should be considered, as these can significantly affect the polyphenol composition of the extract and thus the desired effects. Luță *et al.* [69] showed that the use of biofertilizers and phytosociological cultivation increased the polyphenol content in the leaves of peppermint and lemon balm and altered the content of the individual components, which ultimately changes the antioxidant potential of the extracts. It has also been shown in red wines that the different cultivation parameters have an impact on the total and individual polyphenol concentration, highlighting the need to take cultivation parameters into account when using plant extracts [70].

To date, the potential use of wine extracts as ingredients for various applications has been the subject of numerous studies. In this context, different extracts have been investigated regarding partial aspects relevant to their use in skin care, such as antioxidant [33] and antibacterial [71] properties, as well as influencing the activity of various enzymes [64] [72]. These extracts were often obtained by further extractions and processing steps of seeds [31], stems [64] or skins [32]. In our study, we have shown that the wine extract, a by-product of wine production, already possesses numerous beneficial properties that can be useful in skin care without extensive further processing of the wine pomace. This demonstrates the high economic and environmental efficiency when used in dermal formulations, for example. Furthermore, with our studies on dermal HaCaT cells, we present a holistic analysis of a wine extract highlighting many different aspects and properties, thus emphasizing the enormous benefits for skin care.

5. Conclusion

This study demonstrates the potential of polyphenol-rich wine extract, a by-product of wine production, for use in skin care. Due to the positive influence of the investigated wine extract on elastase inhibition as well as the reduction of reactive oxygen species, it could prevent skin aging. Additionally, the wine extract has been shown to inhibit the growth of pathogenic organisms, potentially supporting a healthy skin microbiome. Furthermore, it was shown that the enzyme lipase is strongly affected by the investigated wine extract, hence lipolysis may be regulated.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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