Chapter

The Beauty and the Toxic Beast: Use of Comet Assay to Study Antigenotoxicity of Natural Ingredients

Sara Gonçalves and Isabel Gaivão

Abstract

The natural cosmetics market has grown since consumers became conscious of natural-based ingredients. A significant number of cosmetics have noxious and chemically potent substances. Thus, the use of natural and organic cosmetics has become increasingly important. An intense investigation into the benefits fruits and plants can bring to our health is required. A healthy lifestyle can reduce these problems, including the consumption or use of substances that protect the genome through various mechanisms that reduce DNA damage. Genotoxicological studies are essential to know the threats to the genome and health, and antigenotoxicological studies are the answer to minimise the instability of the genome. Natural ingredients such as Almond (*Prunus dulcis*), Elderberry (*Sambucus nigra*), Olives (*Olea europaea*), and Grapes (*Vitis vinifera*) have been shown to possess a variety of biological activities and to hold therapeutic promise. They are the most common ingredients in the Trás-os-Montes region (Portugal). This study aimed to demonstrate, *in vivo*, the genotoxicological effects of Elderberry, Almonds, Olives, and Grapes in *Drosophila melanogaster* using the Comet assay.

Keywords: almonds, antigenotoxicity, comet assay, cosmetics, elderberry, genotoxicity, grapes, natural ingredients, olives

1. Introduction

What is your morning routine? You most likely shower with an invigorating shower gel, some shampoo, and a conditioner with a lovely smell. You also apply a hair mask because you have dry and split hair ends. While the mask sets, you scrub your face with a cleanser; you probably shave if you are a man. Then you apply some facial toner and while it dries, use deodorant under your arms and moisturiser to the rest of your body. Next, you apply moisturiser to your face and some sunscreen. You also brush your teeth with that fresh toothpaste. If you are a lady, you then probably apply some makeup. And do not forget your perfume. According to the Environmental Working Group study, the average man uses five to seven

personal care products per day, the average woman uses nine to twelve, and the average teenage girl uses seventeen [1].

A quick look at a cosmetic product's ingredients list shows an enormous amount of noxious and chemically potent substances that impact the environment. A Danish Council THINK Chemicals study from 2020 found that 65 chemicals of concern were found in 39 products [2]. Roughly 69 million individual chemicals and/or chemical combinations are used today [3]. This means consumers are exposed to these chemicals, perhaps daily. The health of our body depends directly on external factors such as cold, heat, humidity, pollution, germs, fungi, bacteria, and the food we eat daily. Our emotions and thoughts also contribute to the maintenance of our health. Healthy habits are associated with life in the open air, good nutrition, and restful sleep. Having beautiful, healthy, silky, and soft skin is the consequence of an excellent functioning organism. Poor digestion and the accumulation of toxins or hormonal imbalances reflect their implications on the skin. To take care of our bodies, we can count on the help of various plants to produce various cosmetics. Environmental elements, air pollution, exposure to solar radiation, and the normal ageing process cause cumulative damage to the building blocks of skin: DNA, collagen, and cell membranes.

The market for natural cosmetics has grown since consumers became conscious of cosmetics with noxious and chemically potent substances and the damage they can cause. People worldwide are striving to make their lifestyle cleaner and safer. As governments begin to act against climate change, growing landfill sites, and the threatening energy crises, we cannot help but consider lifestyle changes and rethink our purchasing habits. Organic has become mainstream, and it is no longer about eating organic food or driving a hydrogen-powered car. The natural and organic market was valued at €76.6 billion at a retail sales price in 2020, and the European cosmetic and personal care cosmetic market is the largest market for cosmetic products worldwide. It is expected to grow annually by 9.24% (2021–2025). There were at least 77 scientific innovation facilities in Europe in 2018 dedicated to research concerning cosmetics and personal care, having spent €2.35 billion in research and development [4, 5].

An intense investigation into the benefits that fruits and plants can bring to our health is needed, as with how to use natural ingredients in cosmetics. A healthy lifestyle can reduce these problems, including the consumption or use of substances that protect the genome through various mechanisms that reduce DNA damage. Genotoxicological studies are essential to know the threats to the genome and health, and antigenotoxicological studies are the answer to minimise the instability of the genome. Natural ingredients such as Elderberry (*Sambucus nigra*), Olives (*Olea europaea*), Almond (*Prunus dulcis*), and Grapes (*Vitis vinifera*) have been shown to have a wide range of biological activities and show promise for therapy. They are the most common ingredients in the Trás-os-Montes region (Portugal). This study aimed to demonstrate, *in vivo*, the genotoxicological effects of Elderberry, Almonds, Olives, and Grapes in *Drosophila melanogaster* using the Comet assay.

2. Skin deep: what is beneath the surface?

The skin has an area of about 2 m^2 and represents about 15% of the body's weight, making it weigh between three and four kilos and is, therefore, the largest and heaviest organ in the human body [6]. The skin covers the entire human organism and is essential for life. Depending on the body region, it has variable appearance,

functions, and structure. The skin is a multifunctional organ that provides external coating, thermoregulation, a healthy microbiological environment, and a defence against external aggressions (cold, heat, pressure, pain). It is divided into three layers: epidermis, dermis, and subcutaneous tissue [7], with some of these layers subdivided into further layers, all with specific functions and roles (**Figure 1**).

The epidermis is the most superficial layer of the skin and is in direct contact with the outside. It is stratified and avascularised epithelial tissue and forms the first line of defence against external factors. It subdivides itself into five layers: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (or basal layer). The stratum corneum consists of corneocytes that have migrated up through the skin. After their 30-day life cycle, they start to dry out and flatten, the nuclei disappear, and the corneodesmosomes (a complex protein that holds the corneocytes) begin to break down. At a certain point, the cells start to shed, a process known as desquamation [9]. The dead keratin cells (keratinocytes) reduce the skin's permeability, preventing water loss. Keratinocytes begin life in the base of the epidermis, and as they develop and grow, they move up through the epidermis, pushed up by new cells continuously produced below. They help retain water and form a protective layer that prevents biological, physical, and chemical agents [10]. The epidermis also protects from ultraviolet rays. The sunlight triggers the production of melanin (the brown pigment that defines our skin colour) in the melanocytes located in the

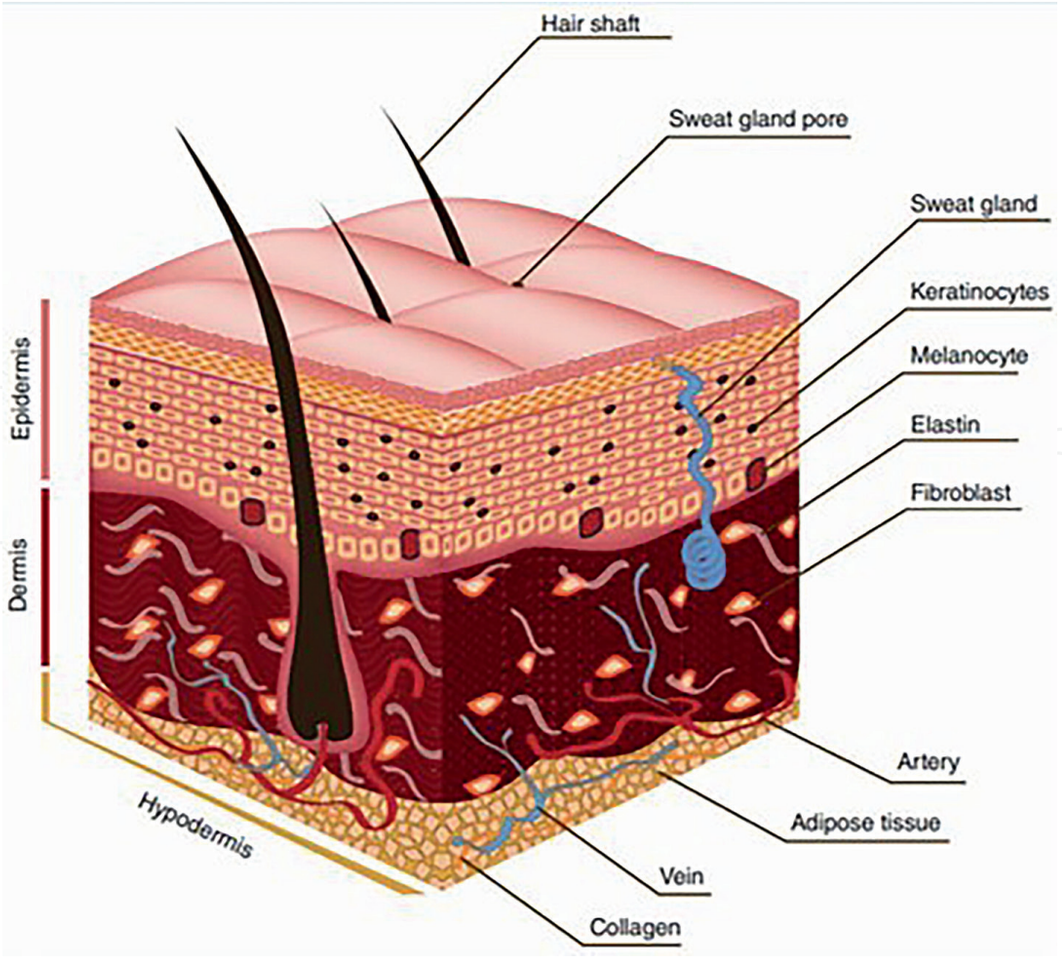

Figure 1.
Representation of the skin. Adapted by Pereira [8].

stratum basale. Melanin acts as a natural sunscreen for the skin and protects it from harmful ultraviolet rays, which is why people get tan when exposed to the sun. But excessive exposure to the sun can disrupt this process, leading to hyperpigmentation. The melanocytes have finger-like projections called dendrites that deliver the melanin into the keratinocytes as they develop and travel up to the stratum corneum [9].

The dermis is a thick, elastic, firm intermediate layer below the epidermis. It subdivides itself into two layers: the reticular layer and the papillary layer [11]. It is composed of irregular connective tissue and has intrinsic collagen fibres, variable elastin fibres, blood vessels, lymphatic vessels, and nerves [10]. It plays an essential role in protecting the body from external and irritating influences, but it also nourishes the upper layers of the skin from the inside. Its thick, firm texture helps to alleviate external pressures. When damage occurs, it contains connecting tissues, such as fibroblasts, that control the production of the extracellular matrix, comprising the structural tissue's collagen and elastin along with water-binding glycoproteins, such as hyaluronic acid [9, 12].

The subcutaneous tissue, which envelops all the muscles except the skin muscles, provides passage for cutaneous nerves, blood, and lymph vessels and plays a role in connecting the dermis and fascia of the muscles [13]. The skin acts like a mesh allowing small molecules to pass freely but screening out larger substances. A substance with a molecular weight or size less than 1000 g/mol can penetrate the skin; with a molecular weight of 400 g/mol, it can enter the cell; with a molecular weight of less than 100 g/mol, it can enter the bloodstream [3]. Smaller materials can easily pass through membranes, enter into DNA, and alter various biochemical functions that are out of our control.

2.1 How and why does the skin's appearance change with age?

Ageing cannot be reversed or even slowed down. However, well-designed cosmetic products can influence the appearance that these changes produce.

The early 20s is when the changes begin. The skin cell turnover starts to slow down gradually. In the 30s, processes that slow down are the natural desquamation, cell turnover, and the rate of collagen production in the dermis. In the 40s, the thickness of the epidermis and dermis decreases, synthesis of collagen and elastin continues to decrease, lipid production slows down, and the glycoproteins begin to decline. The surface becomes more uneven, and fine lines and wrinkles appear as structural integrity declines in the dermis. By the 50s, all processes continue to decline, along with the change in the distribution of subcutaneous fat, the emergence of age spots, and uneven pigmentation. By this stage, all changes lead to apparent effects, such as wrinkles and sagging [9, 14, 15].

The ageing of the skin is primarily associated with the intrinsic genome, as well as with ethnicity (African-American skin is more compacted than Caucasian skin, as well as having a higher intercellular lipid content, which may contribute to more resistance to ageing), anatomical variations (there are significant differences in skin thickness concerning body site, ranging from 0.05 to 0.1 mm on the eyelids to more than 6 mm on the soles of the feet), and hormonal changes in cutaneous tissues (dramatic hormonal changes, particularly thyroid, testosterone, and oestrogen, alter epidermal lipid synthesis) [9, 16, 17]. This type of ageing is known as intrinsic skin ageing.

Alongside this natural ageing process can produce additional ageing effects. These are known as extrinsic ageing factors [17]. These are the ones that each

individual can most influence through changes in their lifestyle and the adoption of a good skincare routine.

Skin is affected by ambient conditions such as temperature and humidity. Low temperature stiffens skin and decreases evaporative water loss even with plenty of moisture in the air, as structural proteins and lipids in the skin are critically dependent on temperature for appropriate conformation [18].

Smoking tobacco has been shown to harm the skin. Those who smoke have fewer collagen and elastin fibres in the dermis, which cause the skin to become slack, hardened, and less elastic. Smoking was an independent risk factor for premature wrinkling even when age, sun exposure, and pigmentation were controlled [19].

The effects of sunlight on the skin are profound and are estimated to be responsible for up to 90% of visible skin ageing, known as photoaging [20]. Sunlight includes three different types of radiation: UVC, UVB, and UVA. UVC (100–290 nm) is primarily blocked by the ozone layer and has little effect on the skin. UVB (290–320 nm) penetrates only into the epidermis, being responsible for the erythema associated with a sunburn. UVA penetrates deeper into the skin and may be responsible for most chronic skin damage associated with photoaging [9, 20]. Ultraviolet radiation is a complete carcinogen, as it initiates cancer through DNA mutation and promotes cancer growth through the inflammatory processes inherent in cumulative ultraviolet exposure [21].

Pollution has become a popular topic regarding premature visible skin ageing. It has been shown that cellular senescence and skin ageing are closely regulated and connected and can be induced by air pollution [22].

3. What is a cosmetic?

The word *cosmetic* comes from the Ancient Greek word *kosmos*, their word describing *the art of dress and ornament* [23]. Ornament is used to describe the outer appearance, and letting many believe that cosmetic products are somehow trivial or lacking depth; many other believe that cosmetic product just applies to makeup. However, makeup, often called colour cosmetics or decorative cosmetics, is one class of cosmetic products. **Figure 2** gives an example of the multitude of cosmetic products covered.

According to the definition of the European Regulation, the term cosmetics signifies a product applied to the body to maintain the skin and, thus, the body as a whole, in good condition, to protect it from the environmental influences and ageing processes, to change its appearance, and to enhance the smell of the body [24]. The definition of cosmetics includes shampoos, soaps, toothpaste, colour cosmetics, hair dyes, cleansing and moisturising creams for regular care, styling products, fragrances, and preparation for protection against ultraviolet light (UV light) [25]. Natural, conventional, and organic cosmetics share the same definition but have different specificities. Certified natural and organic ingredients do not need to be present in the formulation of conventional cosmetics [26]. At least one ingredient derived from a natural substance obtained directly from a mineral or a plant must be contained in a natural cosmetic product and must not be produced synthetically. Natural cosmetics may contain percentages of organic ingredients. Nevertheless, natural products are not necessarily organic [27]. An organic cosmetic must contain at least 95% certified organic ingredients. These raw materials are obtained through approved cultivation and extraction. They must be biodegradable and have as natural

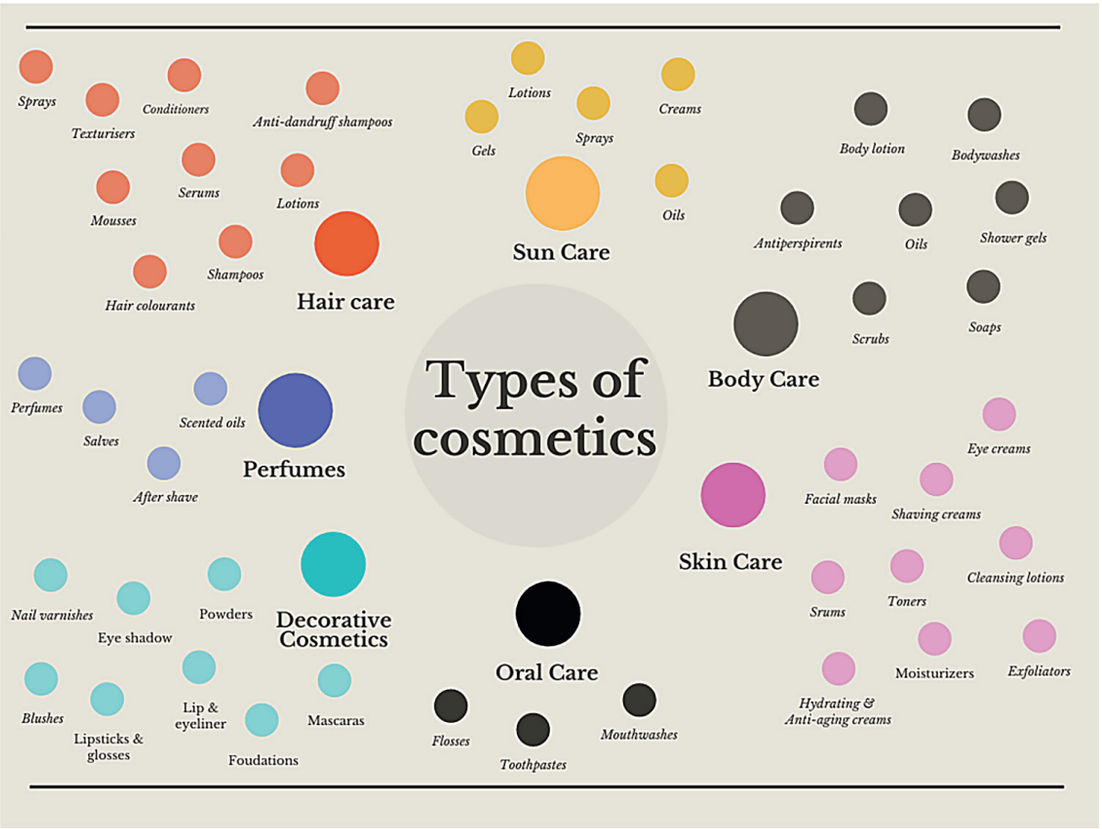

Figure 2.
Diagram showing the range of types of cosmetic products organised and colour-coded in their families.

a chemical nature as possible. The remaining 5% of the formulation may consist of water and natural raw materials from agriculture or non-certified extracting agents approved for organic formulations [25–27].

4. The use of *Drosophila melanogaster* in genotoxicological studies

D. melanogaster was used to evaluate the genotoxic effects caused by natural ingredients exposure. This model organism has become one of the most popular in studies of biological phenomena such as development, neurodegenerative diseases, behaviour, and genotoxicity studies due to its advantages [28–31]. For example, its short life cycle, high prolificacy, and easy handling make it a valuable tool in population studies as it allows the study of several generations in a short period. In addition, their high prolificacy makes statistical analysis easy and reliable [28, 32]. On the other hand, its simple genome, easy manipulation during all phases of development, and the rapid identification of mutants make it a suitable organism in genotoxicity studies, comparative genomics, and evolution [31, 33, 34]. However, the demographic parameters of ageing, such as survival and specific mortality, are highly susceptible even to minor environmental and experimental design variations. Thus, it is necessary to maintain rigorous laboratory practices and carefully control the genetic background to obtain the robust and reliable measurements [35].

The use of *D. melanogaster* as an experimental organism in studies that involve toxicity and genotoxicity has a good level of extrapolation of the results to humans. Due

to the similarities of the detoxification mechanisms, if a compound is antigenotoxic in *D. melanogaster,* it is most likely to be also in humans [36, 37].

5. The comet assay

The alkaline (pH > 13) Comet assay is a sensitive and rapid method for detecting DNA strand breaks in single cells. It is used in genotoxicological studies to determine oxidative DNA damage occurring in various health conditions (in combination with certain bacterial enzymes), to show the protective effects of various nutritional factors in chemopreventive studies, to determine sequence- or gene-specific damage and repair (in combination with fluorescence *in situ* hybridisation) as well as for possible diagnoses [38, 39]. It has the advantages of identifying DNA damage at the single-cell level, sensitivity for detecting low levels of DNA damage using a small number of cells per sample (<10.000), it has a low cost and ease of application, and a short time needed to perform this assay and eukaryote single cell population can be both *in vivo* and *in vitro* [39, 40].

6. Genotoxicity of streptonigrin

Streptonigrin (SN, CAS no. 3930-19-6) is an aminoquinone antitumor antibiotic isolated from cultures of *Streptomyces flocculus* [41]. Due to the potential use of SN in clinical chemotherapy, the study of its genotoxicity is of considerable practical importance. SN inhibits the synthesis of DNA and RNA, causes DNA strand breaks after reduction with NADH, induces unscheduled DNA synthesis and DNA adducts, and inhibits topoisomerase II [42, 43]. This antibiotic causes chromosome damage at the chromosome level and increases the frequency of sister-chromatid exchanges [41]. SN shows its potential to induce a significant level of genotoxicity without the toxic effects (at 20 μM), making it a suitable genotoxic insult for this assay [44, 45].

7. Materials and methods

7.1 Chemicals

Instant Carolina Drosophila Medium Formula 4–24® (hereinafter referred to as Instant Drosophila Medium—IDM) was purchased from Carolina Biological Supply Company, Burlington, USA. Streptonigrin (CAS 3930–19-6) was purchased from Santa Cruz Biotechnology Inc. of Texas, USA. All other chemicals were purchased from Sigma-Aldrich Chemical Company (Madrid, Spain).

7.2 Natural ingredients harvesting and preparation

Elderberry flowers and berries, Olives, Olive Tree Leaves, Grapes, and Almonds were selected in the Trás-os-Montes region, Portugal. This region is bordered by the province of Minho to the west, the Douro region to the south, the Douro River to the east, and Spain to the north. Elderberry is widespread in the north of Portugal, especially in the Varosa Valley, which offers a favourable microclimate for developing this species due to the surrounding mountains [46, 47]. The almond tree is one of the

most widespread tree crops in the Trás-os-Montes region, covering an area of 19,206 hectares. The most widespread varieties are *Parada*, *Casanova*, *Verdeal*, and *Pegarinhos* [48, 49]. Portugal has a wine-growing area of 1/4 to 1/5 of the surface of the important wine-growing countries of Europe. Of the 343 grape varieties listed, about 230 are considered native to Portugal or the Iberian Peninsula, reflecting the extensive and unique Portuguese viticultural genetics [50]. Trás-os-Montes is the second most important Portuguese olive-growing area, currently accounting for between 12 and 15% of national olive oil production. The most important varieties are *Cobrançosa*, *Madural,* and *Verdeal* [51, 52]. In the Trás-os-Montes region, 40 native varieties are grown [50]. Therefore, natural ingredients are easy to obtain in this area. It is also the region with the most organic farmers, and the climatic, topographical, and pedological differences predestine this region for agricultural diversity [53].

Almonds (variety *Pegarinhos*), Red Grapes (variety *Touriga Nacional*), Olive Tree Leaves, and Olives (variety *Cobrançosa*) were obtained from organic farmers in October, September, and December 2021. Elderberry flowers and berries were harvested at Vila Verde, Alijó, Portugal (41°21′44.2”N, 7°33′01.9”W) in May 2022 for the flowers and August 2022 for the berries. Before the experiments, natural ingredients were ground with a coffee mill, obtaining particles <2 mm. After that, they were collected in an airless plastic bag and frozen at -18°C until further analysis.

7.3 Drosophila stock

D. melanogaster Oregon K (Ok) strain was chosen since it has a low antioxidant enzymatic activity and is, therefore, more sensitive for this study [44]. For the genotoxicity assay, crossings were made to obtain heterozygous offspring (w/w^+). Flies were kept in an incubator at 24°C and were anesthetised by etherisation when necessary.

7.4 Genotoxicity evaluation

The evaluation of the genotoxic/antigenotoxic effects of these ingredients was carried out through the Comet assay.

7.4.1 Comet assay

The assay was performed based on the described method [54]. Based on the results obtained in a previous study, for each natural ingredient quantity tested (Elderberry: 5 g; Elderberry flower: 10 g; Olive: 10 g; Olive leaf: 1 g; Grape pulp: 10 g; Almond: 10 g; Almond shell: 1 g; each in a final volume of 100 mL of medium) five instar larvae were isolated and placed in a Petri dish to isolate the neuroblasts. In the end, eight microtubes with five brain ganglia each, immersed in Ringer solution, were obtained. The next step was neuroblast maceration, followed by centrifugation, supernatant removal, and the addition of 140 μL of low melting point (LMP) agarose. Two 70 μL of this solution was then placed in a slide precoated with 10% agarose normal melting point (NMP). Each drop was covered with a coverslip, spreading the solution. This process was repeated for each quantity tested. The slides were stored at 4°C for 5 min, and after agarose solidification, the coverslips were removed. The slides were then immersed in cold fresh lysis solution (2.5 M NaCl, 0.1 M ethylenediaminetetraacetic acid disodium salt (EDTA), 0.01 M Tris base, 1% Triton X-100, pH 10). For the

preparation of the lysis solution, the correct amount of all compounds was dissolved in distilled water (less than the final volume), and pH was set to 10 with 10 M NaOH solution) for 1 h at 4°C, after which they were placed in the electrophoresis chamber with their frozen ends to the cathode and without empty spaces among them. The electrophoresis chamber was filled with cold denaturing and electrophoresis buffer until the slides were covered and stored for 20 min. The next step was the electrophoresis in the dark, where a current of 300 mA and a voltage of 25 V (corresponding a 0,8 V/cm) were applied for 20 min. In the end, the slides were washed in PBS for 10 min at 4°C, then in distilled water for 10 min at 4°C, and left to air-dry. The slides were stained with 40 μL of DAPI (1 μg/mL in water) to each gel and covered with a coverslip. The slide analysis was performed using a fluorescence microscope, and the % of tail DNA and the tail length were scored. For this, 50 cells per gel were observed, and each cell was classified from 0 (no tail) to 4 (almost all DNA in the tail) based on the intensity of its tail. The final score (expressed as "arbitrary units" in a range of 0—400) was obtained by multiplying the mean percentage of nucleoids in each class by the appropriate factor according to this formula:

$$\begin{aligned} Genetic\ Damage\ Indicator\,(GDI) = &\left[\left(\%nucleoid\ class\ 0\right)x\,0\right)] + \left[\left(\%nucleoid\ class\ 1\right)x\,1)\right] \\ &+ \left[\left(\%nucleoid\ class\ 2\right)x\,2)\right] + \left[\left(\%nucleoid\ class\ 3\right)x\,3)\right] \\ &+ \left[\left(\%nucleoid\ class\ 4\right)x\,4)\right] \end{aligned}$$

Data were analysed using the software IBM SPSS Statistics (Statistical Package for the Social Sciences, Chicago, IL, USA), version 20. An analysis of variance (ANOVA) was performed, followed by a Tukey test. Differences were considered statistically significant if $p < 0.05$.

The same procedure was used for the streptonigrin challenge. Considering the final medium volume, streptonigrin was added to the IDM, and dissolved in PBS to attain the final concentration of 20 μM. This concentration was selected according to the literature [44]. A schematic representation of the experimental design can be observed in **Figure 3**.

8. Results

The streptonigrin-challenged group exhibited overall increased DNA damage in all ingredients assessed. Flies fed with C and challenged with streptonigrin presented the highest levels of DNA damage, while flies fed with Eb showed the lowest levels in both unchallenged and streptonigrin-challenged groups (**Figure 4**). Regarding the unchallenged group, there are no statically significant differences. As for the SN-challenged group, there are differences between SN with OTL, GP, and Eb. Statically significant differences were observed between Eb with OP, AS, and A.

9. Discussion

In this Comet assay, flies fed with Elderberry showed the lowest levels in both unchallenged and streptonigrin-challenged groups.

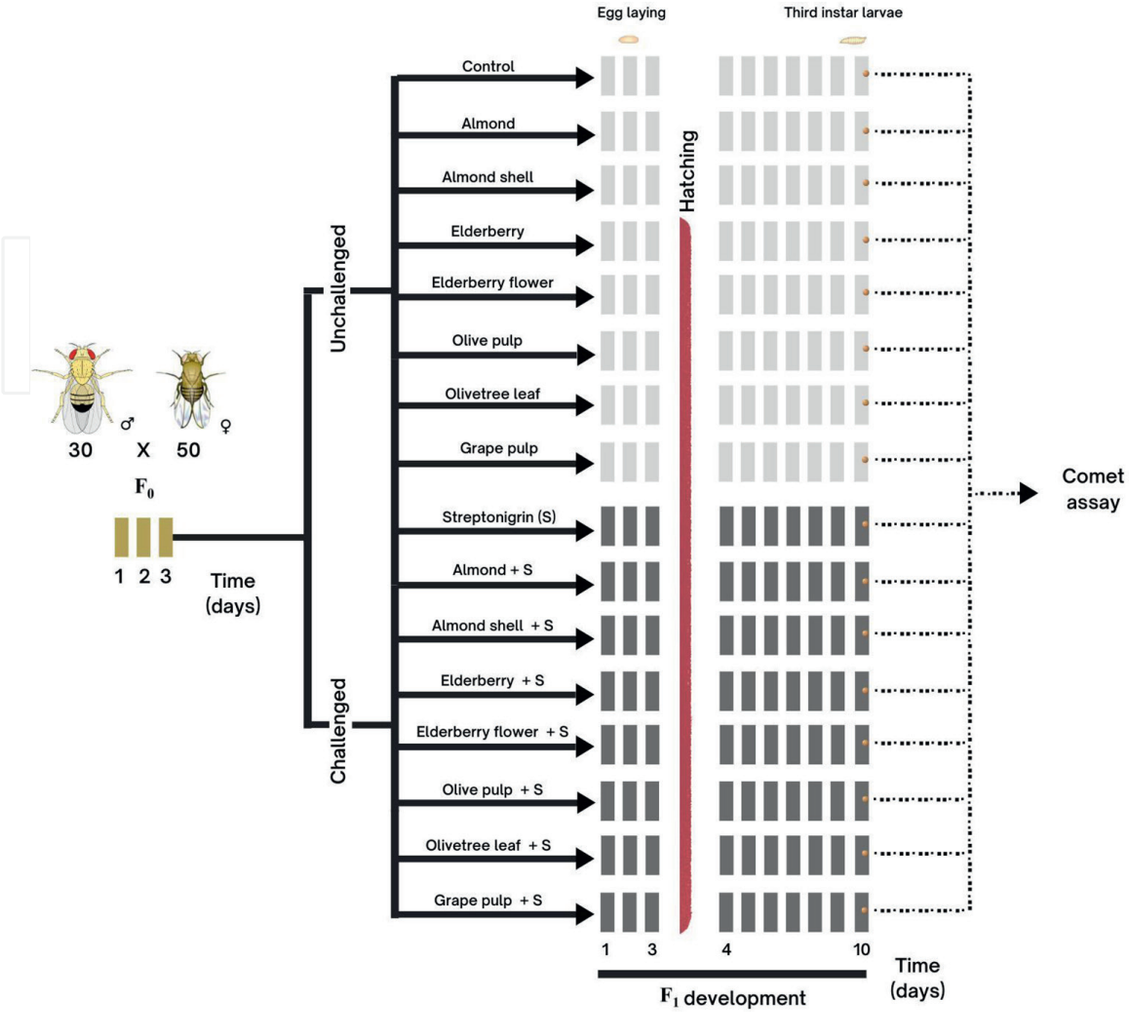

Figure 3.
Schematic representation of the experimental design, elucidating the division of D. melanogaster individuals into two major groups: One unchallenged (light grey time scale) and another streptonigrin-challenged (dark grey challenged) with streptonigrin (SN). The comet assay was performed on larvae in the 3rd instar stage, approximately 10 days after the egg laying.

The identification of ingredients with antigenotoxic effects is one of the most promising areas of research in recent years, as they could protect against DNA damage and its consequences. Studies have shown that antigenotoxic properties are associated with anti-ageing properties [55, 56], and these properties are important in reversing genotoxic effects. Our cells are attacked not only through our skin but also through all the elements we are exposed to. Cosmetics with antigenotoxic properties are capable of neutralising those toxic effects.

10. Conclusions

Beauty is skin deep. The human skin is a powerful organ that seems to be constantly hungry for anything that touches its surface. Oxygen, nitrogen, carbon dioxide, and toxic pollutants enter our skin through three doors: the sweat ducts, hair follicles, and sebaceous glands, or directly through the stratum corneum. This ability

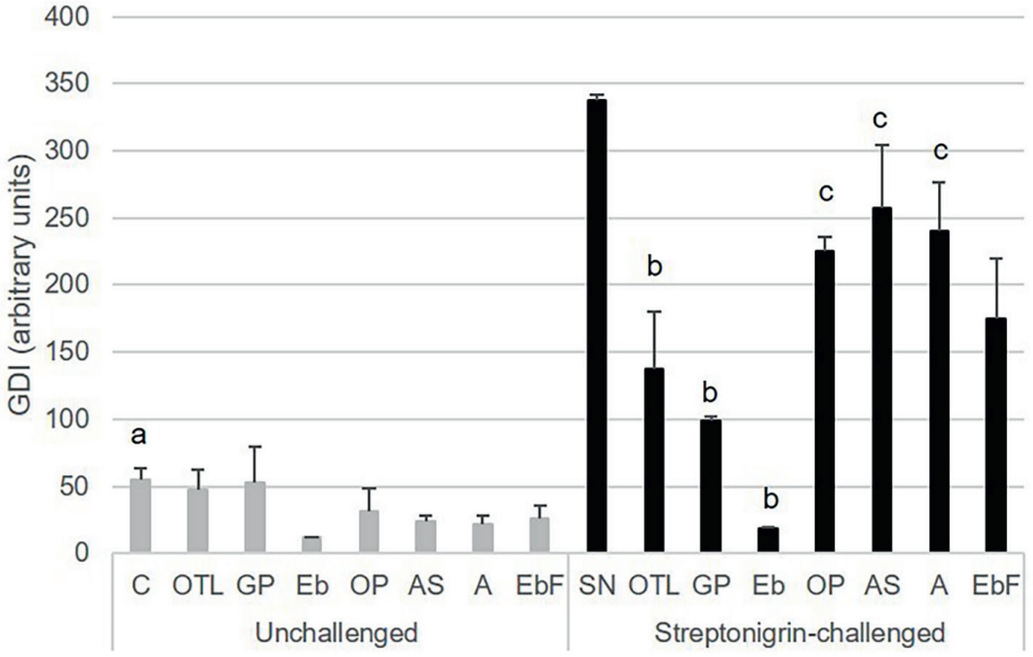

Figure 4.
Mean values of DNA damage (GDI) in drosophila neuroblasts measured by the in vivo comet assay in unchallenged and streptonigrin-challenged groups. Tested groups are identified by abbreviations identifying the ingredient (Eb: Elderberry; OTL: Olive tree leaf; a: Almond; AS: Almond Shell; EbF: Elderberry flower; GP: Grape pulp; OP: Olive pulp). Grey bars correspond to unchallenged groups, and black bars correspond to streptonigrin-challenged groups. Values are mean ± SEM (n = 2). A: Statically significant relative to C; b: Statically significant relative to SN; c: Statically significant relative to BS with treatment.

of the skin to absorb chemical substances so that they can spread throughout the body is often used in medicine. Our skin can absorb up to 64% of substances applied to its surface [57]. Unfortunately, along with water, vitamins, minerals, and oxygen, the skin soaks up potentially carcinogenic ingredients that increase our risk of cancer or other diseases. Natural ingredients hold beneficial properties that need to be studied to be used in the cosmetic industry, benefiting humans. The introduction of antigenotoxic ingredients in cosmetics has the great advantage that, in addition to not being genotoxic per se, and they also neutralise genotoxicity induced by other environmental factors, such as pollution, drugs.

All tested ingredients presented antigenotoxicological properties, with Elderberry having the best results. Such potential cannot be ignored, and further investigation into how to incorporate this ingredient in a cosmetic should occur. Only two studies have been developed since 2012, showing that Elderberry has no mutagenic effect and a high *in vitro* activity [58]. However, none of them focused on cosmetic ingredients.

Additional investigation can be carried out, namely studying antigenotoxicological properties in human lymphocytes. Human lymphocytes are used as surrogate tissue, as they are easily obtained, are available in large numbers, do not require cell culture, are diploids, and are almost all in the same cell cycle phase. Although the Comet assay is well accepted among the scientific community, there are issues regarding standardisation among laboratories [59]. Therefore, new methods for DNA damage assessment would be beneficial to improve research on DNA damage repair and antigenotoxicity.

Acknowledgements

This work was supported by the project UIDP/CVT/00772/2020, funded by the Fundação para a Ciência e Tecnologia (FCT).

The authors would like to thank Paula Santenico and Dinis Gonçalves, organic farmers, for providing the ingredients used in this research.

Conflict of interest

The authors declare no conflict of interest.

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