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In vivo evaluation of Trás-os-Montes natural cosmetic ingredients: Impacts on *Drosophila melanogaster* longevity and genotoxicity

Avaliação *in vivo* de ingredientes cosméticos naturais de Trás-os-Montes: Impactos na longevidade e genotoxicidade da *Drosophila melanogaster*

Evaluación *in vivo* de ingredientes cosméticos naturales de Trás-os-Montes: Impactos en la longevidad y genotoxicidad de *Drosophila melanogaster*

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Abstract
Our body's well-being is intricately linked to external factors, including temperature, humidity, pollution, microorganisms, and the daily food we consume. Nurturing our bodies involves harnessing the benefits of various plants that contribute to the production of diverse cosmetics. Natural elements such as almonds (*Prunus dulcis*), elderberries (*Sambucus nigra*), olives (*Olea europaea*), and grapes (*Vitis vinifera*) have demonstrated a range of biological activities, holding potential for therapeutic applications. This study aimed to assess the *in vivo* impact of these natural ingredients on longevity and genotoxicity/antigenotoxicity in *Drosophila melanogaster*. Longevity was evaluated through regular observations of Drosophila mortality. The somatic mutation and recombination assay were conducted to determine the antigenotoxic potential of the natural ingredients. The findings revealed that these ingredients influenced longevity, and they also exhibited antigenotoxic potential, with elderberry yielding the most promising results. The growing importance of natural and organic cosmetics underscores the significance of identifying ingredients with antigenotoxic effects, offering a promising avenue of research in recent years. Such ingredients could play a crucial role in safeguarding against DNA damage and its subsequent consequences.
Keywords: Antigenotoxicity; Cosmetics; Elderberry; Longevity; SMART assay.

Resumo
A saúde do nosso corpo está diretamente relacionada com fatores externos como temperatura, humidade, poluição, microrganismos e os alimentos que consumimos diariamente. Para cuidar do nosso corpo, podemos contar com a ajuda de diferentes plantas que produzem diversos cosméticos. Ingredientes naturais como amêndoas (*Prunus dulcis*), sabugueiros (*Sambucus nigra*), azeitonas (*Olea europaea*) e uvas (*Vitis vinifera*) têm mostrado uma variedade de atividades biológicas e mostram possuir aplicações terapêuticas. Este estudo teve como objetivo avaliar *in vivo* o efeito desses ingredientes naturais na longevidade e genotoxicidade/antigenotoxicidade em *Drosophila melanogaster*. A longevidade foi avaliada por meio de observações regulares da mortalidade de Drosophila. O ensaio de mutação somática e recombinação foi realizado para determinar o potencial antigenotóxico dos ingredientes naturais. Os resultados mostraram que esses ingredientes induzem alterações na longevidade. Os ingredientes naturais também apresentam potencial antigenotóxico, sendo que o sabugueiro obteve os melhores resultados. O uso de cosméticos naturais e biológicos está a tornar-se cada vez mais importante. Identificar ingredientes com efeitos antigenotóxicos é uma das áreas de pesquisa mais promissoras nos últimos anos, pois poderiam proteger contra danos ao DNA e suas consequências.
Palavras-chave: Antigenotoxicidade; Cosméticos; Sabugueiro; Longevidade; Ensaio SMART.

Resumen
La salud de nuestro cuerpo depende directamente de factores externos como la temperatura, la humedad, la contaminación, los microorganismos y los alimentos que consumimos a diario. Para cuidar de nuestro cuerpo, podemos contar con la ayuda de diferentes plantas que producen diversos cosméticos. Ingredientes naturales como almendras (*Prunus dulcis*), saúco (*Sambucus nigra*), aceitunas (*Olea europaea*) y uvas (*Vitis vinifera*) han demostrado

tener una variedad de actividades biológicas y muestran promesa para la terapia. Este estudio tuvo como objetivo evaluar *in vivo* el efecto de estos ingredientes naturales en la longevidad y la genotoxicidad/antigenotoxicidad en *Drosophila melanogaster*. La longevidad se evaluó mediante observaciones regulares de la mortalidad de Drosophila. Se realizó el ensayo de mutación somática y recombinación para determinar el potencial antigenotóxico de los ingredientes naturales. Los resultados mostraron que estos ingredientes inducen cambios en la longevidad. Los ingredientes naturales también tienen potencial antigenotóxico, siendo el saúco el que arrojó los mejores resultados. El uso de cosméticos naturales y orgánicos está adquiriendo una importancia creciente. Identificar ingredientes con efectos antigenotóxicos es una de las áreas de investigación más prometedoras en los últimos años, ya que podrían proteger contra daños en el ADN y sus consecuencias.
Palabras clave: Antigenotoxicidad; Cosméticos; Saúco; Longevidad; Ensayo SMART.

1. Introduction

Caring for our skin is an integral aspect of a healthy lifestyle. The well-being of our body is directly influenced by external factors such as temperature, humidity, pollution, microorganisms, and the daily food we consume. Additionally, our emotions and thoughts play a role in maintaining overall health (Richman et al., 2005). dopting healthy habits, including exposure to fresh air, balanced nutrition, and restful sleep, is associated with well-being (Kilani et al., 2013). The achievement of beautiful, healthy, silky, and soft skin is a result of well-functioning bodily systems. Conversely, factors like poor digestion, toxin accumulation, or hormonal imbalances can adversely affect the skin (Schagen et al., 2012). To nurture our bodies, we have the support of various plants and foods that yield a diverse range of cosmetics.

In the past, people used ordinary or everyday substances to beautify, heal, soothe or produce other effects on their skin. The first archaeological evidence of the use of cosmetics was found in ancient Egypt around 10,000 BC. Fragrant oils and ointments were made from plants, fruits, seeds, and clay and used by men and women to cleanse and soften their skin and mask body odour (Jain & Chaudhri, 2009). The Egyptians learned the art of distilling essential oils as alternative medicine (Maniche, 1999). In biblical times, the Hebrews used eye cosmetics and perfumed oil for daily and sacred use. One of Job's daughters was named Ker'en-hap'puch, which in Hebrew could mean black paint horn for the eyes, a make-up container or case, perhaps to keep eyeliner for the eyes. Their name could refer to their beauty, but it also suggests that cosmetics were common at that time (Watch Tower Bible and Tract Society of Pennsylvania, 2012). In China, little evidence of the use of cosmetics was found around 3000 BC. Women ingested substances or applied them to the skin to improve their complexion. They used recipes such as bat brain to remove blackheads or powdered dried mandarin peel, white melon seeds, and peach blossom, which were sieved through a sieve and taken thrice daily for 30 days to get beautiful skin (Benn, 2004). In ancient Rome, the most popular preparation was ceruse, a substance made by pouring vinegar over white lead crisps and allowing the lead to dissolve. The mixture was then dried, ground and formed into cakes or tablets that were sold (Olson, 2009). During the Renaissance, pure white and translucent skin was all the rage, and Venetian ceruse was the most commonly used cosmetic. Queen Elizabeth I used ceruse to give her the white appearance she was known for. However, the lead was absorbed through the skin and led to hair loss, muscle paralysis and a slowly deteriorating mental state (Nicolajsen, 2021).

Contemporary cosmetics are progressively incorporating harmful and chemically potent ingredients, causing an ecological impact on the environment. (J. N., 2015). People tend to buy organic and natural products more frequently as awareness grows. Therefore, it is crucial to analyse what benefits plants can bring us. This study aimed to evaluate *in vivo* the effect of the natural ingredients on longevity and genotoxicity/antigenotoxicity in *Drosophila melanogaster*.

2. Methodology

2.1 Chemicals

The Carolina Drosophila Medium Formula 4–24® for immediate use, hereafter referred to as Instant Drosophila Medium (IDM), was procured from Carolina Biological Supply Company in Burlington, USA. Streptonigrin (CAS 3930–19-6)

was sourced from Santa Cruz Biotechnology Inc. in Texas, USA. All remaining chemicals were acquired from Sigma-Aldrich Chemical Company in Madrid, Spain.

2.2 Natural ingredients harvesting and preparation

elected from the Trás-os-Montes region of Portugal, Elderflowers, elderberries, grapes, olives, olive tree leaves and almonds were chosen. This region is situated at the border of Minho to the west, the Douro region to the south, the Douro River to the east, and Spain to the north. The prevalence of elder in northern Portugal, particularly in the Varosa Valley, is attributed to the favorable microclimate created by the surrounding mountains (Braga et al., 2002; Trindade et al., 2019). Trás-os-Montes ranks as the second most significant olive-growing area in Portugal, cultivating 40 indigenous varieties, contributing to 12-15% of the national olive oil production. Key varieties include *Verdea*, *Madural*, and *Cobrançosa* (de Figueiredo et al., 2002; *Portal do INE*, 2013). Comprising a quarter to a fifth of the wine-growing area in important European wine-producing countries, Portugal boasts 343 grape varieties, with approximately 230 being indigenous to Portugal or the Iberian Peninsula, showcasing the nation's extensive and distinct viticultural genetics (Sousa et al., 2007). The almond tree is prevalent in Trás-os-Montes, covering 19,206 hectares, with the most common varieties being *Pegarinhos*, *Casanova*, *Parada*, and *Verdeal*. (Centro Nacional de Competências dos Frutos Secos, 2020; Cordeiro & Monteiro, 2002) The profusion of natural ingredients in the region is enhanced by having the highest concentration of organic farmers, benefiting from climatic, topographical, and pedological variations that make it well-suited for agricultural diversity. (Gonçalves & Gaivão, 2021).

For the study, olive tree leaves, olives (*Cobrançosa* variety), almonds (*Pegarinhos* variety), red grapes (*Touriga Nacional* variety), were sourced from organic farmers in September, October, and December 2021. Elderberries were obtained from INOVTERRA (Vila Pouca de Salzedas, Portugal) in August 2022, while elderflowers were harvested in May 2022 at Vila Verde, Alijó, Portugal (41º21'44.2"N, 7º33'01.9"W). Before conducting the experiments, natural ingredients were meticulously ground using a coffee mill to achieve particles smaller than 2 mm. Following this, they were carefully sealed in airless plastic bags and frozen at -18 ºC until further analysis.

2.3 Drosophila stock

The *Drosophila melanogaster* strain Oregon K (Ok) was chosen due to its low antioxidant enzyme activity, heightened susceptibility to reactive oxygen species (ROS), and, consequently, increased sensitivity, making it well-suited for this study. (Marcos et al., 2014). Individuals of the white Ok strain were used for the longevity analysis. Two different alleles for the sex-linked white (*w*) gene were involved: wild-type with red eyes (*w+*) and mutant with white eyes (*w*). For the genotoxicity test, crosses were made to obtain heterozygous progeny (*w/w+*). The flies were kept in an incubator at 24 ºC and, when necessary, anesthetised by etherisation (with diethyl ether).

2.4 Natural ingredients treatment

For the longevity analysis, three concentrations of ingredients were chosen: 1%, 5%, and 10% (w/v). This assay includes a non-treated standard medium (10 % yeast, 10 % sucrose, 1.2 % agar-agar, 0.5 % propionic acid, and 0.1 % NaCl in water). Fruit flies were kept in 200 mL standard bottles with 30 mL standard medium at 24 ± 1ºC and 60% relative humidity. Each quantity of every ingredient was mixed with the drosophila medium. Thirty couples were mated in each vial with the natural ingredients-treated medium and were allowed to lay eggs. After three days, the flies were disposed of to avoid generation blending. After ten days, the number of males and females of the F_1 progeny was counted.

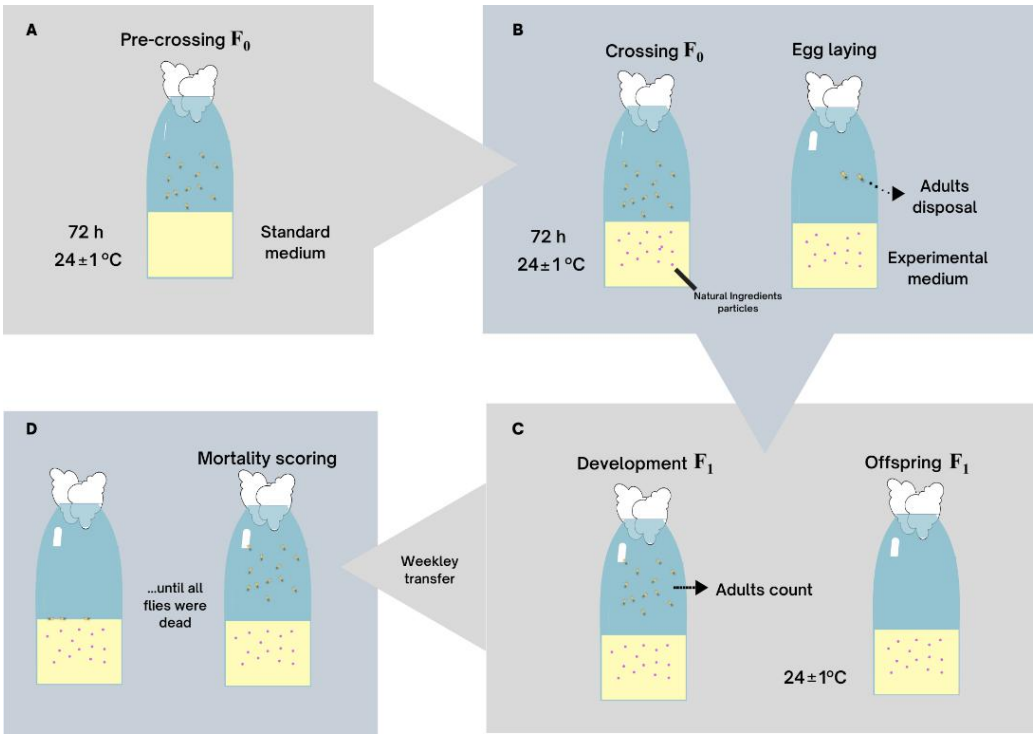
The treatment process remained the same for the genotoxicity assays (SMART), but 50 virgin *w+/w+* females and 30 *w/Y* males were mated. Based on the results obtained from the longevity assay, the best quantity for each natural ingredient

was chosen (elderberry: 5 %; elderflower: 10 %; olive pulp: 10 %; olive tree leaf: 1 %; grape pulp: 10 %; almond: 10 %; almond shell: 1 %).

2.5 Longevity assay

Longevity was assessed through regular observations of drosophila mortality to maintain the best conditions, and the flies were transferred to new vials every week (Figure 1).

Figure 1 - Experimental design of the longevity assay: (a) pre-crossing, (b) transfer of flies, crossing and disposal, (c) development of F_1 progeny (adults were counted) and transfer, and (d) weekly renewal of the medium.



Source: Authors (2022).

2.6 Genotoxicity evaluation

The evaluation of the genotoxic effects of this ingredient was carried out through the SMART assay.

2.6.1 SMART assay

The somatic mutation and recombination assay (SMART) was used to analyse genotoxicity. This assay aims to assess the induction of genetic damage in somatic cells of adult flies after larval exposure by using genetic markers observed phenotypically in adult tissues to detect loss of heterozygosity (LOH) in heterozygous or transheterozygous individuals and the resulting expression of two recessive alleles, indicating the occurrence of point mutations, deletions, mitotic recombination or/and nondisjunction. In this study, the eye *w*/*w*+ SMART was chosen, and the X chromosome "white" gene (*w*) was used as a recessive marker to observe, in wild-type eyes, the presence of white clones in the ommatidia indicating the occurrence of LOH (Marcos et al., 2014).

For each quantity (elderberry: 5 %; elderflower: 10 %; olive pulp: 10 %; olive tree leaf: 10 %; grape pulp: 10 %; almond: 10 %; almond shell: 1 %), 50 *w*+/*w*+ and 30 *w*/Y males, were crossed in standard medium for three days, to obtain heterozygous progeny. After the eclosion, the adult flies were counted and isolated. The scoring was performed through eye analysis on females under the stereoscopic microscope and cold light. Males only present white eyes, and due to this, it wasn't

possible to calculate the recombination effect, which would be obtained by the difference between the number of spots found in females and the number found in males since the latter could not be due to homologous recombination. The flies were observed at ×40 magnification, and the eyespots were counted. At least 400 eyes per concentration were analysed.

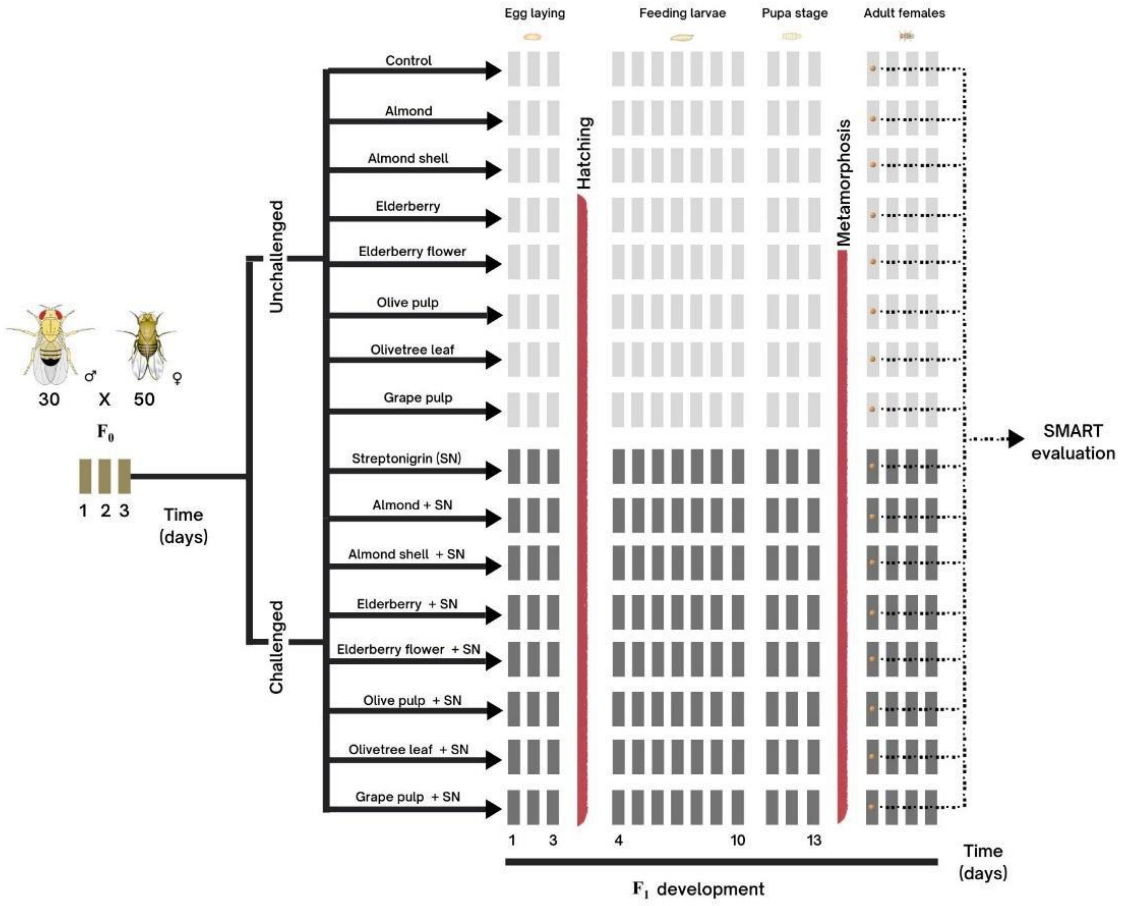
For the diagnostic statistical analysis between treatments, a double decision approach using the χ^2 test for proportions was performed, thus allowing the response to be classified as positive (+), weakly positive (w+), inconclusive (i), or negative (-) (Frei & Würgler, 1988; Gaivão & Comendador, 1996).

The percentage of inhibition (IP) was calculated as the percentage of decrease in patch frequency under the different conditions with ingredient supplementation (without challenge) compared to the control (without supplementation; C), as described by Abraham (Abraham, 1994), using the following formula:

$$IP = \frac{control - condition\ with\ ingredient}{control}\ x\ 100$$

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 reach the final concentration of 20 μM. This concentration was chosen following the literature (Gaivão & Comendador, 1996). A schematic representation of the experimental design is shown in Figure 2.

Figure 2 - Schematic representation of the experimental design illustrating the pre-crossing of *Drosophila melanogaster* (3 days) and the subsequent development of the F_1 generation, in which the SMART procedure was applied to adult females after metamorphosis. The pairs were divided into two large groups: one untreated (light grey time scale) and one treated (dark grey time scale) with streptonigrin (SN).



Source: Authors (2022).

2.7 Statistical analysis

Descriptive statistics included the mean and standard error of mean (SEM) for continuous variables. The Kaplan-Meier method was used to analyse the survival distribution of the flies, and a Log-Rank test was used to compare the survival distribution among groups with different ingredient quantities (Bland & Altman, 2004; Han et al., 2016; Winters et al., 2010). Survival plots were presented when significant differences in survival between groups were deemed. Data were analysed using GraphPadTM Prism 9 and OASIS 2 for survival analysis. A p-value inferior to 0.05 was considered statistically significant.

2.8 Ethical statement

Animal welfare ethical review boards do not have to approve experimental settings applying the Drosophila melanogaster, so our study did not require ethical board approval because it did not contain human or other vertebral animal trials (Baenas & Wagner, 2019)-

3. Results

3.1 Effects on longevity

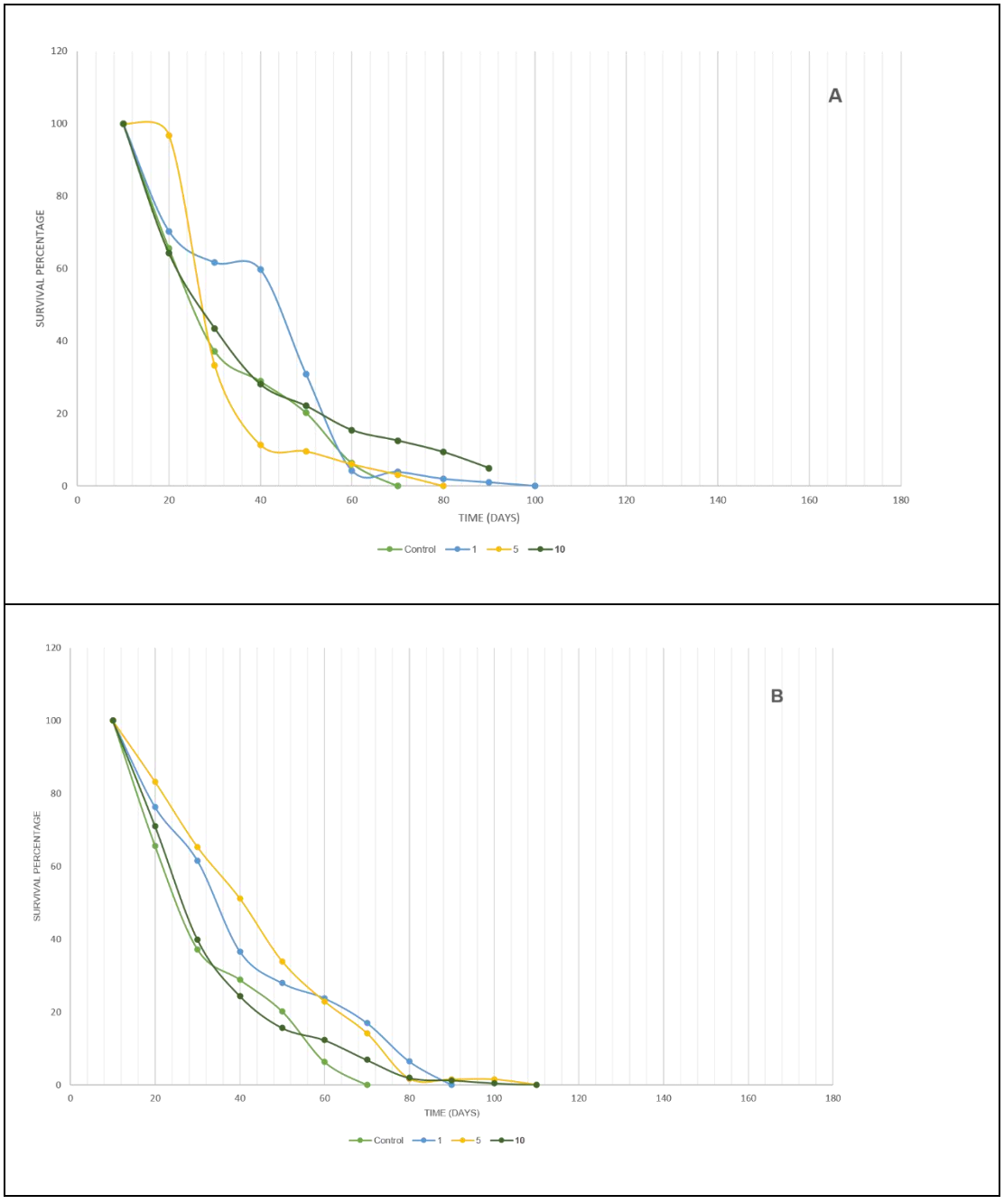
Table 1 shows the survival analysis results regarding mean survival time and the survival distribution comparison between groups. There was a decreased mean survival time trend with increasingly higher ingredient quantity. Flies fed with 1 % of almond shell (Figure 3A), 10 % of almond (Figure 3B), 1 and 10 % of elderflower (Figure 3 C), 5 % of elderberry (Figure 3D), 10 % olive pulp (Figure 3E), and 10 % of grape pulp (Figure 3F) exhibited a significantly different survival distribution when compared with the control groups.

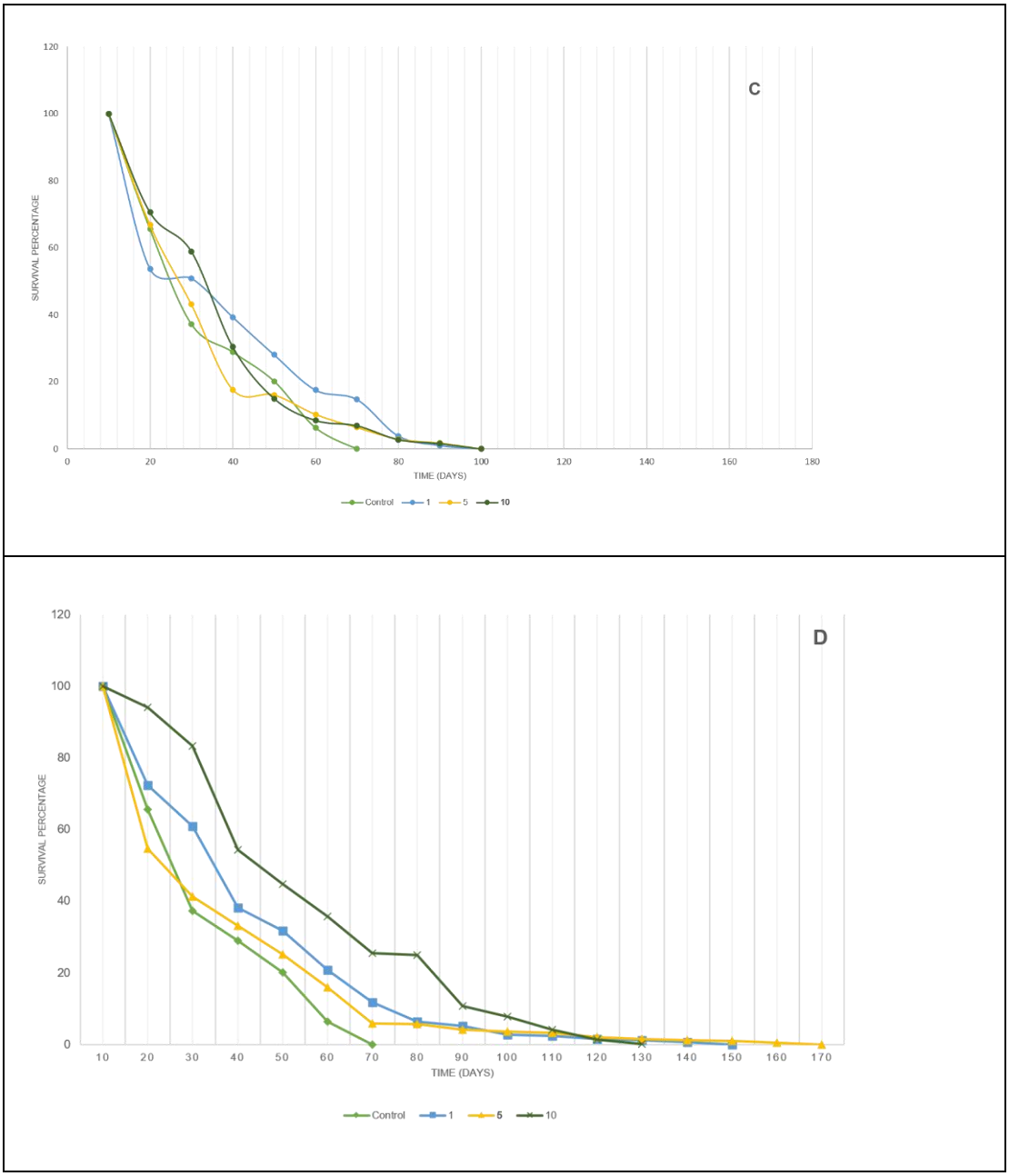
Table 1 - Statistical analysis of the longevity assay of all of the natural ingredients. The Kaplan-Meier method was used to analyse the survival distribution of the flies, and a log-rank test was used to compare the survival distribution among groups with different ingredient percentages. The significance level was set at 0.05 (bold).

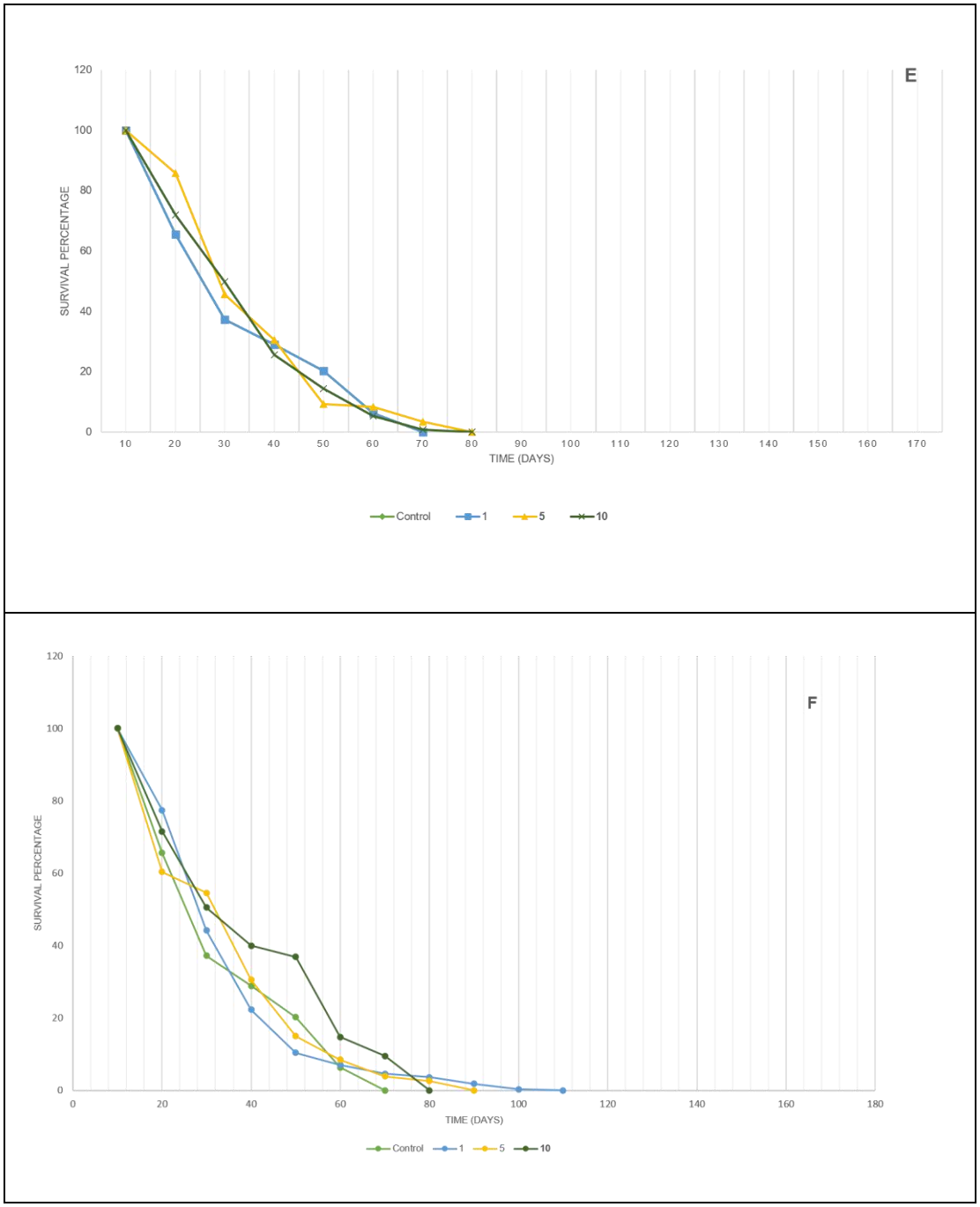
Ingredient	Percentage	Mean survival (days)	SEM	Log-rank test p-value*
Almond Shell	**Control**	35.81	0.71	-
	1 %	39.53	0.66	**<0,001**
	5 %	36.03	0.74	0.6522
	10 %	43.35	0.81	NC
Almond	**Control**	35.81	0.71	-
	1 %	44.30	1.3	NC
	5 %	47.51	0.96	NC
	10 %	37.32	0.75	**0.0247**
Olive Tree Leaf	**Control**	35.81	0.71	-
	1 %	35.97	1.20	0.3819
	5 %	43.40	1.37	NC
	10 %	47.15	1.30	NC
Olive Pulp	**Control**	35.81	0.71	-
	1 %	38.28	1.03	0.2988
	5 %	39.77	0.89	1
	10 %	38.28	1.03	**0,0002**
Elderberry	**Control**	35.81	0.71	-
	1 %	45.57	0.93	NC
	5 %	39.45	0.85	**0.0029**
	10 %	57.12	0.86	NC
Elderflower	**Control**	35.81	0.71	-
	1 %	36.5	0.83	**<0,001**
	5 %	39.47	0.82	0.3911
	10 %	36.50	0.83	**0.0039**
Grape Pulp	**Control**	35.81	0.71	-
	1 %	37.16	0.94	**1**
	5 %	37.27	1.4	1
	10 %	41.37	1.94	**0.0002**

Source: Authors (2022).

Figure 3 - Survival plot of flies fed with different quantities of almond shell (A), almonds (B), elderflower (C), elderberry (D), olive pulp (E), and grape pulp (F). These ingredients exhibited a significantly different survival distribution compared to the control groups. Each colour represents a different quantity (Blue: Control; Orange: 1 %; Grey: 5 %; Yellow: 10 %). The quantity with the best result is marked in bold (A: 1 %; B: 10 %; C: 1 and 10 %; D: 5 %; E: 10 %; F: 10 %).




C
SURVIVAL PERCENTAGE
TIME (DAYS)
Control
1
5
10
D
SURVIVAL PERCENTAGE
TIME (DAYS)
Control
1
5
10



Source: Authors (2022).

3.2 Genotoxic effect

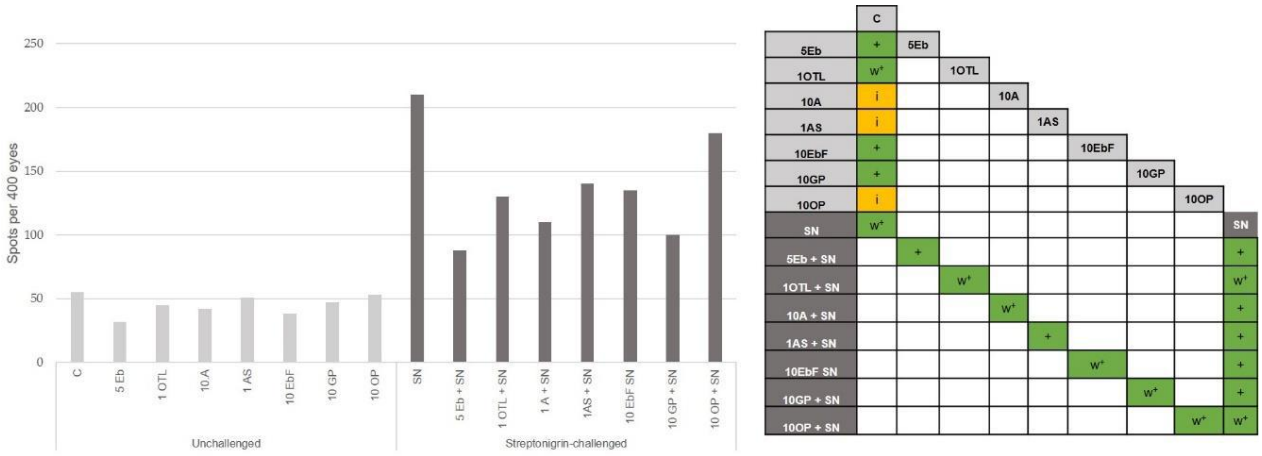
3.2.1 SMART assay

Concerning unchallenged groups, the spot frequency of all ingredients is lower relative to the control group (Figure 4). Elderberry (5Eb) exhibited the most promising result as this ingredient decreased the number of total spots compared to the control.

All the conditions challenged with SN significantly increased the number of spots relative to the unchallenged conditions. In addition, the spots frequency in all groups challenged with SN and supplemented with the ingredient (5Eb+SN, 10OTL+SN, 10A+SN, 1AS+SN, 10Eb+SN, 10GP+SN, and 10OP+SN) significantly decreased relative to the non-supplemented group (SN). In both groups (unchallenged and streptonigrin-challenges), elderberry had the lowest number of spots/400 eyes. The χ^2 multiple-decision (m=2, i.e., two times) showed a positive outcome regarding C for 5Eb, 10EbF, and

10GP. When comparing the unchallenged group with the SN-challenged, regarding these ingredients, 5Eb+SN versus 5Eb has a positive outcome, 10EbF+SN versus 10EBF, and 10GP+SN versus 10GP have a weak positive result. When comparing 5Eb with SN, this ingredient has a positive result.

Figure 4 - Frequency of spots (spots per 400 eyes) in the eyes of *Drosophila melanogaster* (SMART). The table shows the statistical diagnosis performed by a double decision approach using the χ^2-test for proportions for the spot frequency comparisons (only comparable conditions are shown; +, positive result; w+, weak positive result; -, negative result; i, inconclusive result), according to Frei & Würgler (Frei & Würgler, 1988). Abbreviations denote the groups tested (Eb: elderberry; OTL: olive leaf; A: almond; AS: almond shell; EbF: elderflower; GP: grape pulp; OP: olive pulp); '+ SN' means that streptonigrin was incorporated into the medium; C (no supplementation with any ingredient) and SN (no supplementation with any ingredient plus SN exposure) were used for comparison purposes. The light grey columns (in the graph) and cells (in the table) correspond to the groups with no streptonigrin exposure; the dark grey columns and cells correspond to the groups with streptonigrin exposure. The ingredients were included in the Instant Drosophila Medium in a percentage (%) shown by the number preceding the letter indicative of the ingredient supplementation in the groups' abbreviation.



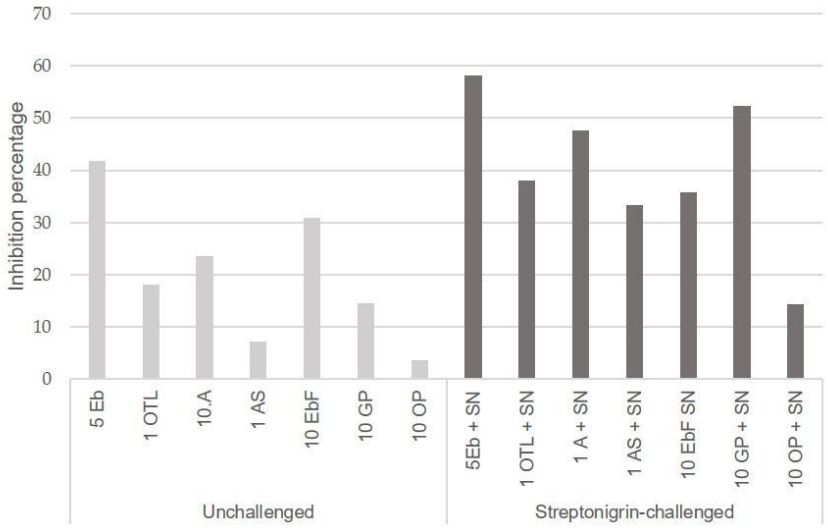
Source: Authors (2022).

To better assess the effect of ingredient addition, the inhibition percentage (IP) was calculated for both challenged and unchallenged conditions (Figure 5). Higher IP was verified in elderberry (41,81%), followed by elderflower (30,91%).

Increasing IPs were verified for all challenged conditions, as well as unchallenged ones. Concerning the SN-challenged conditions, the highest IP was obtained by elderberry (58,10%), followed by grape pulp (52,38%), and almonds (47,62%).

Higher IPs were identified in all ingredients in the SN-challenges condition than in the unchallenged one.

Figure 5 - Inhibition percentages of the SMART conditions. IP is calculated as the percentage of decrease for spot frequency in the different conditions with ingredients supplementation (without insult) compared to the control. Abbreviations denote the groups tested (5 Eb, 1 OTL, 10 A, 1 AS, 10 EbF, 10 GP, and 10 OP), which are derived from the percentage (%) of ingredient supplementation and the abbreviations identifying the ingredient (Eb: elderberry; OTL: olive tree leaf; A: almond; AS: almond shell; EbF: elderflower; GP: grape pulp; OP: olive pulp), with the addition of '+SN' to indicate the streptonigrin incorporation in the medium. The light grey bars correspond to unchallenged groups, and the dark grey bars correspond to streptonigrin-challenged groups.



Source: Authors (2022).

4. Discussion

The *in vivo* evaluation of the biological effects of natural cosmetic ingredients, such as elderberry, elderflower, almond, almond shell, olive tree leaf, olive pulp, and grape pulp, from the Trás-os-Montes region of Portugal in *D. melanogaster* is a critical step of a significant pathway to obtain suitable conclusions to be extrapolated to humans. This present research aimed to evaluate, *in vivo*, the effect of the natural ingredients on longevity and genotoxicity/antigenotoxicity in *D. melanogaster*.

In *D. melanogaster*, supplementation with the natural ingredients during larval development increased mean longevity. Elderberry showed more significant potential to promote longevity than other natural ingredients. Flies fed with 5 % elderberry survived up to 170 days, compared to the control group, which lived only up to 70 days. Longevity reflects good cellular function throughout the body, indicating antigenotoxicity and genome stability. It would also be helpful to test intermediate quantities of elderberry, as supplementation with 10 % of elderberry led to statistically non-significant results. Further testing would clarify the specific quantity of elderberries required for the longevity-enhancing actions.

The significant spot reduction for all SN-challenged conditions supplemented with the natural ingredients (compared with the non-supplemented one) showed the potential of the natural ingredients in counteracting basal DNA damage and the genotoxicity developed by SN. Elderberry (challenged and un-challenged) had fewer spots/eyes (32 and 88, respectively). With the double-decision test, elderberry had a positive outcome in every situation, compared with the control, the SN-challenged, and only SN. Regarding its inhibition percentage in unchallenged and challenged groups, elderberry had the highest rate (41,81% and 58,10%, respectively), meaning this ingredient would act as an antigenotoxic/antimutagenic.

SN, an aminoquinone antitumor antibiotic, is derived from cultures of *Streptomyces flocculus* (Bolzán & Bianchi, 2001). Given the potential application of SN in clinical chemotherapy, investigating its genotoxicity holds significant practical importance. SN exhibits various modes of action, including the inhibition of DNA and RNA synthesis, induction of DNA

strand breaks upon reduction with NADH, stimulation of unscheduled DNA synthesis and DNA adduct formation, and inhibition of topoisomerase II (Cohen et al., 1963; Kim et al., 1998). This antibiotic induces chromosome-level damage and elevates the frequency of sister chromatid exchange (Bolzán & Bianchi, 2001). Remarkably, SN can induce a substantial level of genotoxicity at 20 μM without toxic effects, rendering it an ideal genotoxic agent for this assay (Gaivão et al., 1999; Gaivão & Comendador, 1996). In this context, elderberry may exhibit its antigenotoxic potential by enhancing the antioxidant mechanisms of fruit flies, acting as dietary antioxidants, and shielding the flies from genomic damage caused by SN.

Studies have shown that elderberry has a high antioxidant (Dawidowicz et al., 2006; Genova & Lenaz, 2015; Sidor & Gramza-Michałowska, 2015) and anticancer activity (Pan et al., 2012) due to its high content of vitamin C and anthocyanins. Using elderberries may constitute a potential protective agent against growth and unfavourable effects of oxidative stress in the human body (Dawidowicz et al., 2006; Sidor & Gramza-Michałowska, 2015). Functional decline associated with ageing is primarily related to oxidative damage to the genome (Genova & Lenaz, 2015). That being the case, antioxidant mechanisms are necessary to delay ageing. Applying products that contain antioxidants can provide excellent antioxidant protection (Pan et al., 2012). The potential to promote longevity and antigenotoxicity displayed by elderberry could be achieved by scavenging ROS, directly donating electrons and/or protons to enzymatic and/or endogenous non-enzymatic antioxidants for ROS conversion.

4.1 Limitations

While *D. melanogaster* is a valuable model organism due to its genetic tractability and short lifespan, its physiological differences from humans might limit the direct extrapolation of findings to human biology. Further validation in mammalian models or human studies is necessary to confirm these effects in a more relevant context. The study primarily focused on specific concentrations of natural ingredients. A broader concentration range analysis could provide a more nuanced understanding of dose-dependent effects and unveil potential biphasic or differential responses at varying concentrations. The study primarily investigated longevity and genotoxicity/antigenotoxicity. Expanding the assessment to include other relevant endpoints, such as inflammatory markers, oxidative stress parameters, or gene expression profiling, could offer a more comprehensive evaluation of these natural ingredients' effects. The study utilised whole extracts of natural ingredients, which contain a myriad of compounds. Identifying and isolating specific bioactive compounds responsible for observed effects may be challenging, requiring more targeted investigations.

5. Conclusion

The demonstrated antigenotoxic and longevity-promoting properties of natural ingredients, particularly elderberry, elderflower, almonds, almond shells, olive pulp, grape pulp, and olive tree leaves, hold significant promise for the cosmetic industry. Incorporating these natural components into cosmetic formulations could yield multifaceted benefits. Natural ingredients rich in antioxidants, such as elderberry and grape pulp, could be potent ingredients in anti-aging skincare products. Their ability to scavenge ROS and mitigate DNA damage could improve skin health and reduce signs of ageing, like wrinkles and fine lines. Given their demonstrated antigenotoxic potential, these natural extracts may be utilised in developing genoprotective cosmetics. Products safeguarding the skin against environmental pollutants, UV radiation, and other genotoxic agents could leverage these ingredients for enhanced protective effects. With an increasing consumer preference for natural and organic cosmetics, incorporating these natural ingredients can cater to this growing market demand. Products boasting naturally derived components associated with health benefits could drive market competitiveness and consumer loyalty.

Beyond cosmetic applications, these findings hold implications for broader human health. Identifying natural ingredients exhibiting antigenotoxic effects benefits cosmetic formulations and underlines their potential contributions to

overall skin health and wellness. Such ingredients may aid in preventing or mitigating skin conditions associated with DNA damage, inflammation, and oxidative stress. While studying in the context of cosmetics, these natural ingredients might have systemic health benefits beyond skin care. Their antioxidant and antigenotoxic properties suggest potential nutraceuticals or dietary supplement applications to promote overall health and reduce the risk of age-related diseases.

Further research aimed at delineating the underlying molecular mechanisms behind the observed effects of these natural ingredients is crucial. Understanding how these compounds interact with cellular pathways, modulate gene expression, or influence specific biochemical pathways will enhance their potential applications. Conducting well-designed human trials or clinical studies to validate the effects observed in *Drosophila melanogaster* is essential. Investigating these natural ingredients' topical or systemic application in human subjects, assessing their safety profiles, and exploring long-term effects on skin health and general well-being will provide valuable insights. Expanding the concentration range analysis to examine the dose-response relationships of these natural ingredients would give a clearer understanding of their optimal concentrations for beneficial effects while avoiding potential adverse effects. Efforts to isolate and identify specific bioactive compounds within these natural extracts responsible for their effects should be intensified. Isolating individual compounds and assessing their efficacy independently could lead to developing more targeted and potent formulations. Further exploration into formulation development, stability studies, and compatibility assessments of these natural ingredients in different cosmetic formulations will pave the way for their successful integration into commercial products. Also, conducting comprehensive safety evaluations, including skin sensitisation tests, irritation studies, and long-term toxicity assessments, is imperative before commercialising these ingredients.

While this study provides valuable insights into the potential benefits of natural ingredients on longevity and genotoxicity/antigenotoxicity, further investigations addressing the outlined limitations and embarking on suggested future research avenues will strengthen these findings' scientific validity and translational potential.

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