

In vivo developmental and antioxidant effects of some *Salvia* species on *Drosophila melanogaster*

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Abstract

Medicinal plants have long served as valuable sources of therapeutic agents, primarily due to their antioxidant and antitumor properties. Turkey, known for its rich biodiversity and diverse climatic conditions, hosts approximately 10000 plant species with nearly 400 possessing medicinal potential. Among these, species of the genus *Salvia* are particularly prominent, traditionally used to treat a wide range of ailments including gastrointestinal, respiratory, cardiovascular, renal and cognitive disorders. In this study, it is the first time that the developmental toxicity and *in vivo* antioxidant activities of aqueous methanolic, ethanolic and ethyl acetate extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* were evaluated using *Drosophila melanogaster* (larvae, pupae and adult). Developmental toxicity was assessed by measuring percentage of pupation, eclosion and overall survival. *In vivo* antioxidant activity was determined using DPPH, ABTS and FRAP assays. The results indicated that none of the extracts of *S. suffruticosa* except the methanolic extract affected the pupation and survival rates ($p > 0.05$), while other plant extracts negatively influenced the normal developmental processes. All extracts of *S. suffruticosa* demonstrated high *in vivo* antioxidant capacities (≥ 80 % activity) according to the ABTS test, while methanolic and ethanolic extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis* and *S. syriaca* showed high antioxidant activities according to DPPH test (> 50 - 70 % activity). FRAP test also showed that the Trolox equivalent antioxidant capacities reported here for *S. multicaulis* (0.0025 – 0.0060 $\mu\text{g TE/g}$) and *S. suffruticosa* (0.0049 – 0.0061 $\mu\text{g TE/g}$) indicate strong antioxidant potential. To conclude the results of the *in vivo* antioxidant activity, it is demonstrated in this study that the studied plant extracts didn't harm the physiological antioxidant system (balance) of the

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whole organism and the studied *Salvia* species have strong potential for the development of effective natural antioxidant supplements.

Keywords: *Salvia*, developmental toxicity, in vivo antioxidant activity, *Drosophila melanogaster*

Bazı *Salvia* türlerinin *Drosophila melanogaster* üzerindeki in vivo gelişimsel ve antioksidan etkileri

Öz

Tıbbi bitkiler, özellikle antioksidan ve antigenotoksik özellikleri nedeniyle uzun zamandır değerli terapötik ajan kaynakları olarak hizmet vermiştir. Zengin biyoçeşitliliği ve farklı iklim koşullarıyla bilinen Türkiye, yaklaşık 400'ü tıbbi potansiyele sahip yaklaşık 10.000 bitki türüne ev sahipliği yapmaktadır. Bunlar arasında, geleneksel olarak gastrointestinal, solunum, kardiyovasküler, böbrek ve bilişsel bozukluklar dahil olmak üzere çok çeşitli rahatsızlıkları tedavi etmek için kullanılan *Salvia* cinsine ait türler özellikle öne çıkmaktadır. Bu çalışmada, ilk defa *Salvia candidissima*, *S. multicaulis*, *S. suffruticosa* ve *S. syriaca*'nın su, metanol, etanol ve etil asetat özütlerinin gelişimsel toksisitesi ve in vivo antioksidan aktiviteleri, model organizma olarak *Drosophila melanogaster* (larva, pupa ve ergin) kullanılarak değerlendirilmiştir. Gelişimsel toksisite, pupalaşma yüzdesi, eklozyon yüzdesi ve genel sağkalım yüzdesi ölçülerek değerlendirilmiştir. In vivo antioksidan aktivite, DPPH, ABTS ve FRAP analizleri kullanılarak belirlenmiştir. Sonuçlar, *S. suffruticosa*'nın metanolik özütü dışında hiçbir özütünün pupalaşma ve yaşama oranlarını etkilemediğini ($p > 0,05$), diğer bitki özütlerinin ise normal gelişim süreçlerini olumsuz etkilediğini gösterdi. *S. suffruticosa*'nın tüm özütleri ABTS testine göre yüksek in vivo antioksidan kapasiteler ($\geq$ %80 aktivite) gösterirken, *S. candidissima* subsp. *candidissima*, *S. multicaulis* ve *S. syriaca*'nın metanol ve etanol özütleri DPPH testine göre yüksek antioksidan aktiviteler ($>$ %50-70 aktivite) gösterdi. FRAP testi ayrıca, burada *S. multicaulis* (0.0025-0.0060 $\mu\text{g TE/g}$) ve *S. suffruticosa* (0.0049-0.0061 $\mu\text{g TE/g}$) için bildirilen Trolox eşdeğer antioksidan kapasitelerinin güçlü antioksidan potansiyeli gösterdiğini gösterdi. In vivo antioksidan aktivite sonuçlarını özetlemek gerekirse; bu çalışmada, incelenen bitki ekstraktlarının tüm organizmanın fizyolojik antioksidan sistemine (dengesine) zarar vermediği ve incelenen *Salvia* türlerinin etkili doğal antioksidan takviyelerinin geliştirilmesi için güçlü bir potansiyele sahip olduğu gösterilmiştir.

Anahtar kelimeler: *Salvia*, gelişimsel toksisite, in vivo antioksidan aktivite, *Drosophila melanogaster*

1. Introduction

Medicinal plants have been important natural medicine sources used for the prevention or exposure of diseases since ancient times. The protective effect of many plant species and herbs is thought to be due to the presence of antioxidant and antigenotoxic components in the tissues of the plants [1], [2]. Turkey is considered one of the important

countries in the world in terms of plant genetic resources due to its different climate and topographic features and has around 10 thousand plant species. In this richness, medicinal and aromatic plants have great importance. Approximately 400 plants in Turkey's flora have the potential for medical use. *Salvia* species have an important place among these plants [3].

Salvia species have been used in folk medicine to treat stomach complaints, influenza, colds, coughs, cardiovascular disorders, renal disorders, digestive problems, fractured bones, burns and memory-loss [4]. In literature there are some studies about the phytochemical content, antioxidant, antimicrobial and cytotoxic effects of different *Salvia* species. For example, essential oil analyses of *Salvia candidissima* showed that spathulenol, camphor, caryophyllene oxide and borneol are found in high levels [5], [6]. In addition, methanolic and ethanolic extracts of *S. candidissima* subsp. *candidissima* have been reported to have strong antioxidant activity and to have significant antifungal properties against some fungal pathogens. In addition, studies on mericarp and seed extracts have shown that *S. candidissima* has rich sterol, amino acid and mineral content [7]. Some cytotoxicity tests also revealed the apoptosis-inducing effects of *Salvia candidissima* Vahl. subsp. *candidissima* on breast cancer cells [8]. Pehlivan et al. investigated the total antioxidant /oxidant status and oxidative stress index of *Salvia multicaulis* and found that the plant had a strong antioxidant effect [9]. Free radical scavenging effects of compounds (lupeol, β -sitosterol, stigmasterol, caffeic acid and 1-feruloyl- β -D-glucopyranose) isolated from methanol, chloroform and petroleum ether extracts prepared from aerial parts of *S. suffruticosa* were evaluated by DPPH method and it was reported that caffeic acid provided the highest activity with IC₅₀ value of 12 μ g/mL [10]. In another study, the antimicrobial effect of essential oil obtained from aerial parts of *S. suffruticosa* was investigated by the disk diffusion method and reported that essential oil exhibited significant activity against *Staphylococcus saprophyticus*, *Salmonella typhi*, *Shigella flexneri* and *Escherichia coli* but was not effective against *S. aureus* and *S. epidermidis* [11]. Researchers also found that the essential oil of *Salvia syriaca* showed cytotoxicity, antioxidant, α -amylase/glucosidase inhibitory activities thanks to its rich phytochemical content (spathulenol, isospathulenol, bornyl acetate, rutin, quercetin, apigenin, ferulic acid and rosmarinic acid) [12]. While these studies in the literature have revealed important properties of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* species, there are no studies on the developmental toxicity and in vivo antioxidant activities of plant extracts. This study will make a unique contribution to this gap in the literature.

A crucial component of hazard assessment for environmental chemicals and pharmaceuticals is the review of the developmental toxicology data [13]. Developmental toxicology reveals the effects of environmental factors on survival while following the embryological development of the organism. In this sense, *Drosophila melanogaster* is used as an important model organism. *Drosophila melanogaster* is a powerful model organism widely used in genetics, developmental biology, and toxicology studies. It is particularly preferred in toxicity studies due to its well-defined genetic structure, short life cycle, low maintenance costs, high offspring production, and similarity to many human genetic pathways. Furthermore, despite being an invertebrate, *Drosophila* can yield results similar to mammalian systems in assessing processes related to oxidative stress, aging, neurodegeneration, and environmental toxins. In a study published by Tuz et al. (2025), the effectiveness of *D. melanogaster* as an intermediate model for

toxicology research was highlighted and it was stated that it offers a suitable platform to obtain reliable results before moving on to mammalian models [14]. Similarly, in the study by Fiala et al. (2025), it was stated that the behavioral and physiological responses of both larval and adult *Drosophila* to environmental toxins and pesticides are well defined, making it a reliable model for the analysis of various toxicological parameters [15]. For these reasons, the *D. melanogaster* model was used in our study and was preferred because it offers both ethical and practical advantages over alternative systems.

Another parameter is the examination of *in vivo* antioxidant activities. It is obvious that many plant species have antioxidant capacities. However, if the issue is to demonstrate its usability as a supplement, *in vitro* tests alone cannot prove this. The first step in tracking antioxidant supplementation is to check host antioxidant levels to make sure the supplement has been absorbed [16]. In literature, there are various experiments measured the DPPH level in *Drosophila*, *in vivo* [17], [18]. Solvents such as water, methanol, ethanol and ethyl acetate are standard solvents frequently used in herbal extraction, covering a wide polarity spectrum. Each solvent is suitable for the solubility of different phytochemical groups, allowing extracts to vary in terms of content and biological effects. Water is particularly suitable for polar compounds (tannins and water-soluble polyphenols), while methanol and ethanol are suitable for medium-polar phenolics and flavonoids. Ethyl acetate is suitable for low-polar essential oils and lipophilic compounds. The use of these solvents has enabled comparative toxicological and antioxidant analyses by isolating different bioavailable components of plant material [19]. Therefore in this study, water, methanol, ethanol and ethyl acetate extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* were studied to investigate the developmental toxicity and *in vivo* antioxidant effects on *Drosophila melanogaster*.

2. Methods

2.1. Plant collection and extractions

The *S. multicaulis*, *S. syriaca*, *S. suffruticosa* and *S. candidissima* subsp. *candidissima* species used in the study were selected and collected by Assoc. Prof. Dr. Mikail Açar. The *S. syriaca* and *S. suffruticosa* were collected from Tunceli Center Atatürk neighborhood, empty fields, 900 m (GPS: 39.077585, 39.533380 and GPS: 39.077282, 39.531123). *S. multicaulis* was collected from Tunceli center Aktuluk neighborhood, Munzur University campus open areas and roadsides, 1000 m (GPS: 39.045467, 39.508405). *S. candidissima* subsp. *candidissima* was collected from Tunceli center - Ovacık roadside 5th km, approximately 1000 m (GPS: 39.121075, 39.508863).

Air-dried and powdered leaf and flower material (50 g) was extracted with 250 mL of solvent (water, methanol, ethanol, or ethyl acetate) for each extraction. In water, methanol, and ethanol extracts, the mixture was left to stand overnight at a temperature not exceeding the boiling point of the solvent. For the preparation of the ethyl acetate extract, the material was left in the shaker overnight with ethyl acetate, and interaction with the solvent was supported by three separate 10-minute sonication cycles. After all extracts were filtered through filter paper, the solvents were removed using an evaporator under vacuum, and the resulting oily solid residues were stored at -20°C . These methods were adapted from previously described protocols [20], [21].

2.2. Growth conditions of *D. melanogaster*

Throughout the assays, the *Oregon-R* wild type strains grown in the Molecular Biology and Genetics Department of Balıkesir University were used for the study. The flies were maintained at room temperature $22^{\circ}\text{C} \pm 1$ in an incubator to maintain stable room temperature [22]. In literature, temperatures between 20 to 25°C is accepted as a normal growth temperatures for *D. melanogaster* [23]. Deviations from this range can negatively impact their survival and reproductive capacity. Prolonged exposure to temperatures of 30°C or higher may lead to sterility or death, while temperatures below 18°C can slow down their development and reduce viability [24]. After being generated for multiple generations and inbred, *Oregon-R* strains were used for the tests; they were only taken out of the incubator for the duration of the experiments. As stated in the earlier research, all flies were fed conventional growth media [25], [26]. To prepare the growth medium, 43 g of sugar was dissolved in 500 mL of distilled water at 100°C using a magnetic stirrer. Next, 9 g of agar was added. Once the agar fully dissolved and turned transparent, 90 g of semolina was incorporated. After the semolina particles swelled, the mixture was removed from the heat and allowed to cool at room temperature for 3 minutes. Then, 25 g of yeast was added. Following this, 30 μL of an antifungal agent (Mikostatin, Code 228/97, Deva Holding, Istanbul, Turkey) and 5 mL of propionic acid were introduced and thoroughly mixed. The prepared medium was then portioned into sterile glass culture vials, with 25 g allocated to each [27]. The growth media were supplemented with extract solutions at 50 mg/L concentrations by mixing and the dose was determined according to the previous studies in literature [27], [28], [29].

2.3. Developmental toxicity tests

In vivo developmental toxicity was assessed by monitoring the developmental stages of *D. melanogaster* exposed to the plant extracts [30], [31], [32], [33], [34]. 25 female and 25 male adult *Drosophila* were moved to vials with normal media and cultured to produce larvae. The third-instar larvae (72 ± 4 h) that developed at the conclusion of the period were removed from the medium and divided equally between experimental vials (50 larvae for each vial) that were exposed to the extracts (50 mg/L) and negative control groups that were not. Positive control group was prepared treating the flies with H_2O_2 ($6.5 \mu\text{g mL}^{-1}$). The H_2O_2 dose was chosen according to previous toxicity studies in the literature [1], [27], [29], [35], [36]. For pupae and adult formation, the vials were kept at 22°C , and their progress was tracked. The number of pupae and adults in each group was recorded during the transformation period from larvae to pupae and from pupae to adult. Each group underwent three iterations of the experiment. The following formulas were used to determine the pupation, survival and eclosion percentages [30], [31], [32], [33], [34]:

$$\text{Pupation \%} = \frac{\text{Total pupae}}{\text{Total larvae}} \times 100 \quad \text{Eq. (1)}$$

$$\text{Survival \%} = \frac{\text{Total adult flies}}{\text{Total larvae}} \times 100 \quad \text{Eq. (2)}$$

$$\text{Eclosion \%} = \frac{\text{Total adult flies}}{\text{Total pupae}} \times 100 \quad \text{Eq. (3)}$$

2.4. *In vivo* antioxidant activity tests

In vivo antioxidant activity test was performed according to the previous studies in literature [18], [20]. 50 adult male and female *D. melanogaster* flies were exposed to the growth media containing plant extracts (50 mg/L) and H_2O_2 ($6.5 \mu\text{g/mL}$) to evaluate *in vivo* antioxidant activity and incubated at 22°C for 5 days. The incubation period was

determined by the previous studies in literature [37], [38], [39], [40]. The H₂O₂ dose was chosen according to previous toxicity studies in the literature [1], [27], [29], [35], [36]. The groups were then homogenized in cold phosphate buffer (100 µl of 0.1 M, pH: 7.0) and centrifuged at 4000 g for 10 min [18], [20], [41]. The supernatants were then used for DPPH, ABTS and FRAP tests as indicated below:

The supernatants and 0.6 mM DPPH reagent (10 %, v/v) were mixed. After keeping them in dark for 1h, the absorbances were measured at 517 nm. *In vivo* antioxidant activity of the isolated supernatant from each group was as also determined by ABTS radical scavenging activity method. ABTS⁺ radical solution was prepared using equal volumes of ABTS salt and ammonium persulfate and kept in the dark overnight. For sample analysis, 2.95 mL of ABTS⁺ solution and 0.05 mL of supernatant were mixed and measurements were made at 734 nm with a UV-Visible spectrophotometer.

The *in vivo* antioxidant activities observed by DPPH and ABTS tests were calculated using the Eq. (4).

$$\text{In vivo antioxidant activity \%} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \times 100 \quad \text{Eq. (4)}$$

The *in vivo* antioxidant activities of supernatants obtained from each experimental group were also evaluated using FRAP method. This method was applied according to previously published protocols [42]. The oxidant for this test was prepared using 2.5 mL of 2,4,6-tripyridyl s-triazine dissolved in a solution consisting of 40 mM HCl, 25 mL acetate buffer, and 20 mM FeCl₃ in dH₂O. The final solution contained 1.67 mM Fe(III) and 0.83 mM 2,4,6-tripyridyl s-triazine. In this study, absorbances were taken at 593 nm after plant extracts were added to the oxidant solution. The calibration curve was prepared using Trolox, a hydrophilic analogue of vitamin E. Values are expressed as "mg/mL Trolox".

2.5. Statistics

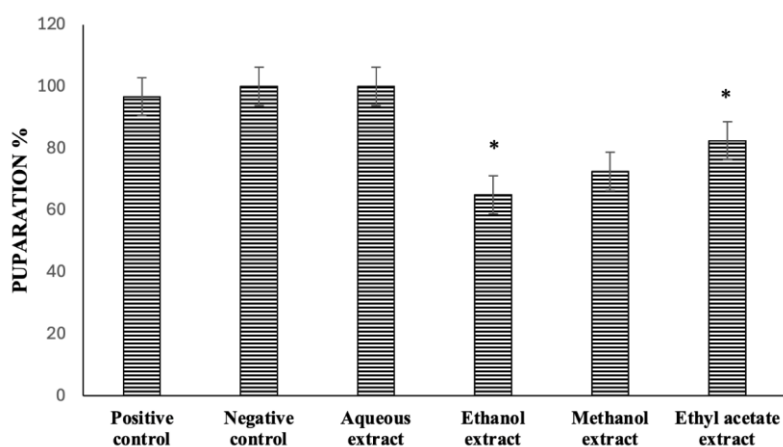
Standard errors (SE) were computed based on the fact that each experiment was conducted in a minimum of three biological replicates. Microsoft Office Excel was used as a tool to apply the student's t-test, and a p-value of less than 0.05 was considered statistically significant.

3. Results and discussion

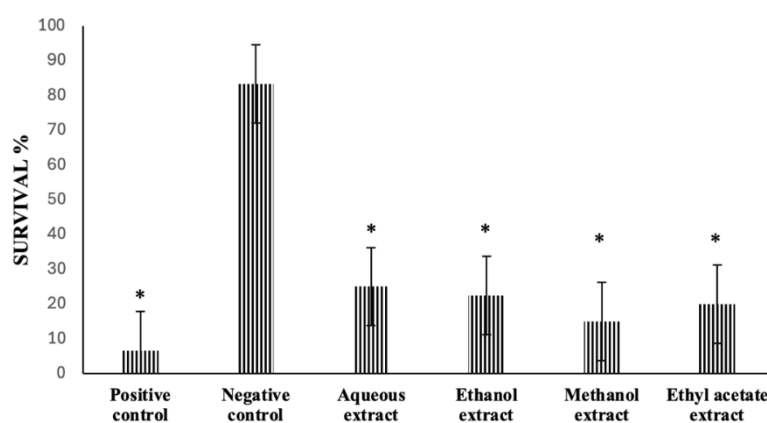
3.1. Developmental toxicity test results

For many years, developmental biologists and geneticists have used the fruit fly (*D. melanogaster*) as a model of choice and recently *Drosophila* has been widely acknowledged as a tool for elucidating the basic genetic processes of hazardous chemicals in toxicology investigations [33]. In this study, puparation, survival and eclosion rates of water, ethanol, methanol and ethyl acetate extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* plants and the effects of hydrogen peroxide (H₂O₂) were evaluated in *D. melanogaster*. Larvae, pupae and adult flies were counted and the experimental results are presented in Figure 1-4. The negative control group exhibited high rates of puparation (100%), survival (83%) and eclosion

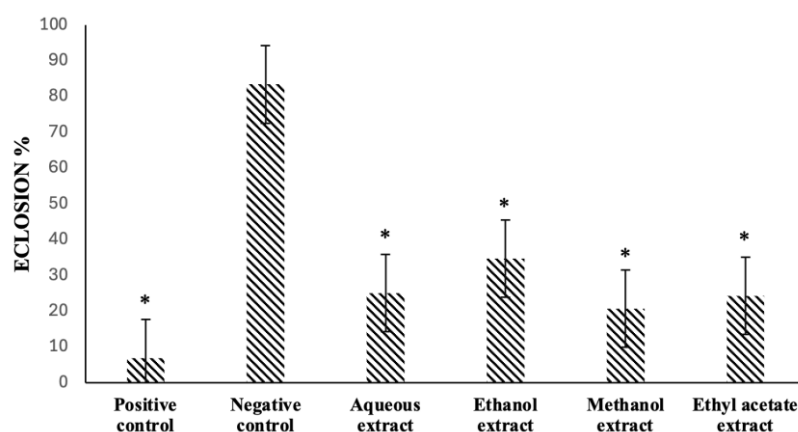
(83%) as an indicator of suitable experimental conditions. On the other hand, H₂O₂, used as a positive control, induced strong oxidative stress and significant decreases in pupuration (97%), survival (7%) and eclosion (7%) rates, confirming the lethal and developmental toxicity of H₂O₂. Although, the decrease in pupuration % after H₂O₂ exposure seems high, the decrease is statistically significant compared to negative control ($p < 0.05$). The reason of high pupuration % may be because of the fact that the high activity of antioxidant enzyme catalase in *D. melanogaster* larvae occurring as a result of oxidative stress load due to high metabolic rate and cellular growth. The catalase gene represents a key component of the antioxidative defense network. In adult flies, catalase levels have been reported to either drop or remain largely unchanged as a function of age. On the other hand, during development catalase levels vary in a dynamic fashion. The level of enzyme activity peaks occur at the same time with ecdysteroid hormone increases which trigger pupariation and the mRNA levels of catalase which is the main enzyme involved in the elimination of H₂O₂ in *D. melanogaster* [43], [44], [45]. The solvents used in this study are known to cause toxic effects in *D. melanogaster* but in this study this affect was eliminated by using evaporation system [46], [47], [48]. The aqueous extract of *S. candidissima* subsp. *candidissima* showed the same level of pupuration rate as the negative control with 100% pupuration but caused significant decreases in survival and eclosion rates (Figure 1). Other extracts of the *S. candidissima* subsp. *candidissima* also caused significant inhibitions on the developmental processes of the flies (Figure 1). *S. multicaulis* and *S. syriaca* extracts also significantly decreased the pupuration, survival and eclosion rates (Figure 2 and 4, $p < 0.05$). None of the extracts of *S. suffruticosa* except the methanolic extract affected the pupuration and survival rates ($p > 0.05$), but they significantly affected the eclosion % values (Figure 3, $p < 0.05$). When the differences between the positive control values and the numerical data of the developmental processes caused by the extracts were compared, it was seen that the *S. suffruticosa* extracts gave much higher values than the positive control among the other plant extracts. In fact, the results indicated that none of the extracts of *S. suffruticosa* except the methanolic extract affected the pupuration (60-80 %, $p > 0.05$) and survival rates (50- 60 %, $p > 0.05$), while other plant extracts negatively influenced the normal developmental processes. This situation clearly showed that the extracts of *S. suffruticosa* did not interfere with the developmental processes. There isn't any study in literature for *in vivo* developmental toxicity screening after the exposure of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* plant extracts in *D. melanogaster* to compare those results however there are some findings with other plant species. For example, researchers found that the supplementation of aqueous leaf extract of *C. orchoides* to *Drosophila* larvae didn't cause gut toxicity but significantly reduced the development, reproduction and survival of the flies [49]. Another study also investigated the effects of different plant volatiles (*trans*-Cinnamaldehyde, benzaldehyde, allyl isothiocyanate, hexanal and ethyl formate) and found that the plant volatiles significantly inhibited larval emergence from the spotted-wing *Drosophila* eggs in artificial diet compared to the control [50]. In another study, *Mangifera indica* aqueous stem bark extracts were tested with *D. melanogaster* and after the exposure the survival-rate, the emergence of young flies, climbing abilities and antioxidant activities of fruit flies significantly decreased with increased concentrations of the *M. indica* extracts [51].



(a)



(b)



(c)

Figure 1. Developmental toxicity test results showing pupation % (a), survival % (b) and eclosion % (c) following exposure with aqueous, ethanol, methanol and ethyl acetate extracts of *S. candidissima* subsp. *candidissima* (*, indicates statistical significance compared to the negative control ($p < 0.05$)).

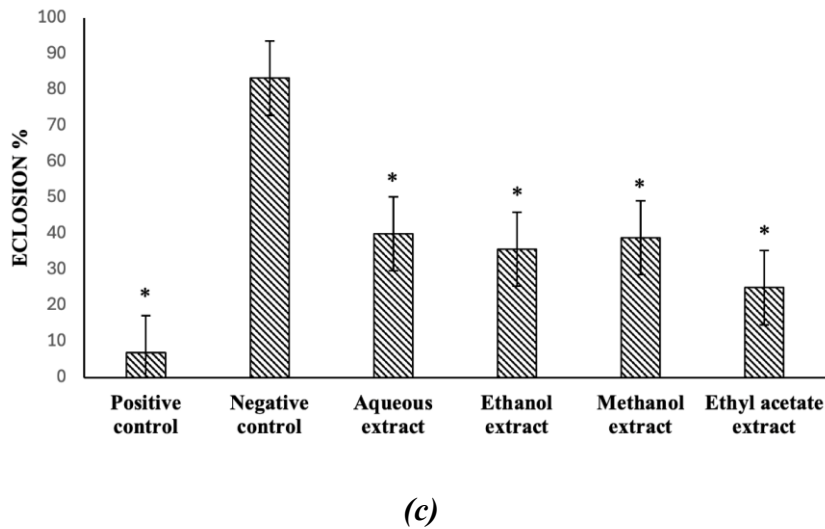
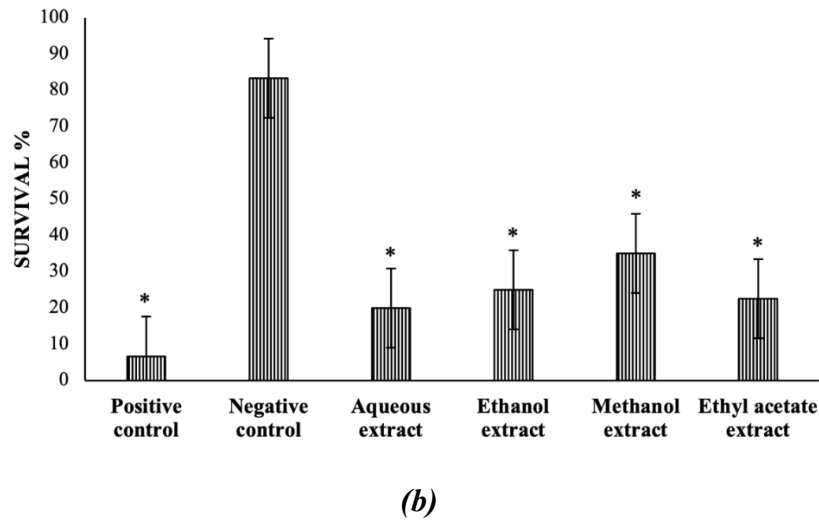
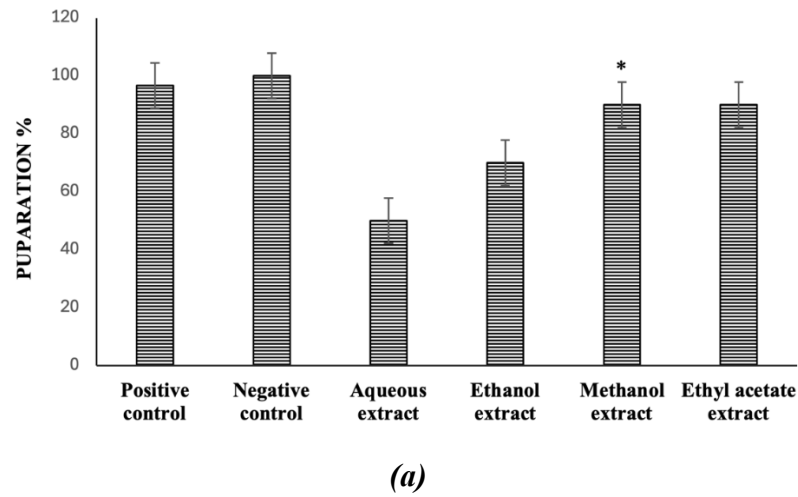
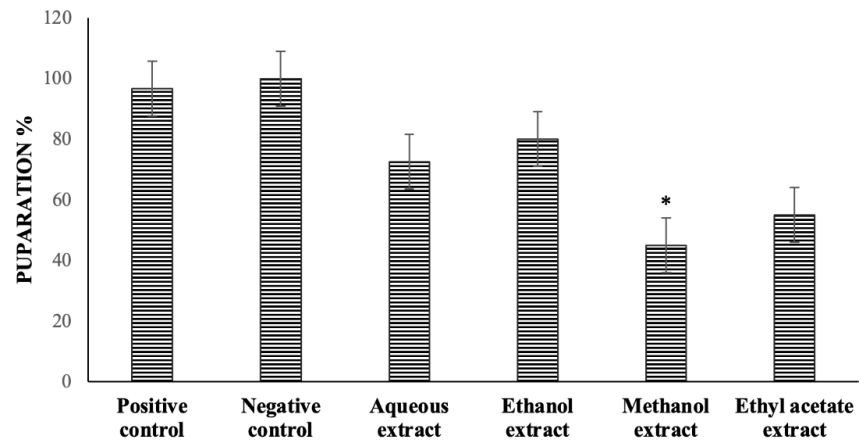
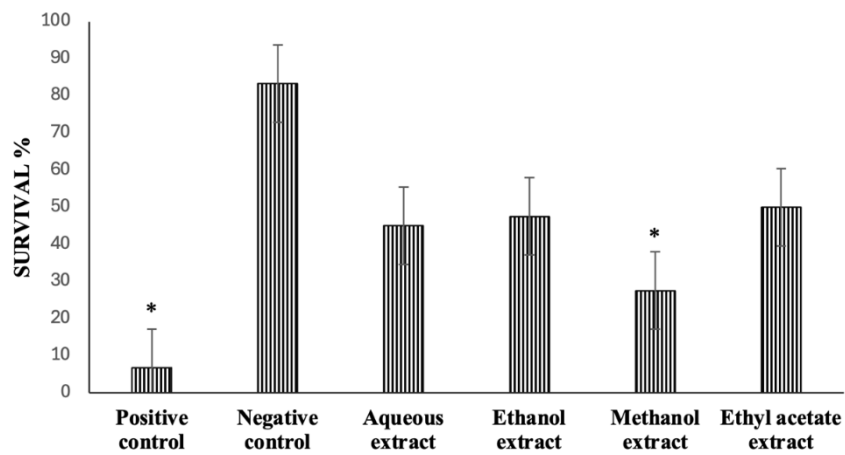


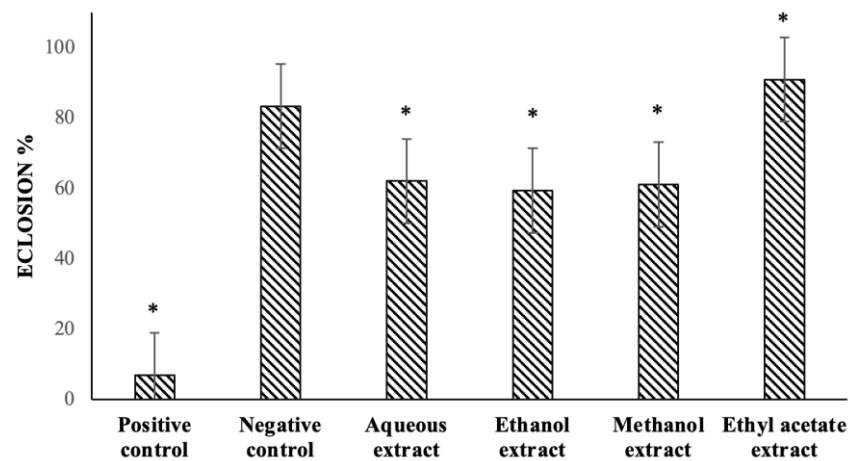
Figure 2. Developmental toxicity test results showing puparation % (a), survival % (b) and eclosion % (c) following exposure with aqueous, ethanol, methanol and ethyl acetate extracts of *S. multicaulis* (*, indicates statistical significance compared to the negative control ($p < 0.05$)).



(a)



(b)



(c)

Figure 3. Developmental toxicity test results showing puparation % (a), survival % (b) and eclosion % (c) following exposure with aqueous, ethanol, methanol and ethyl acetate extracts of *S. suffruticosa* (*, indicates statistical significance compared to the negative control ($p < 0.05$)).

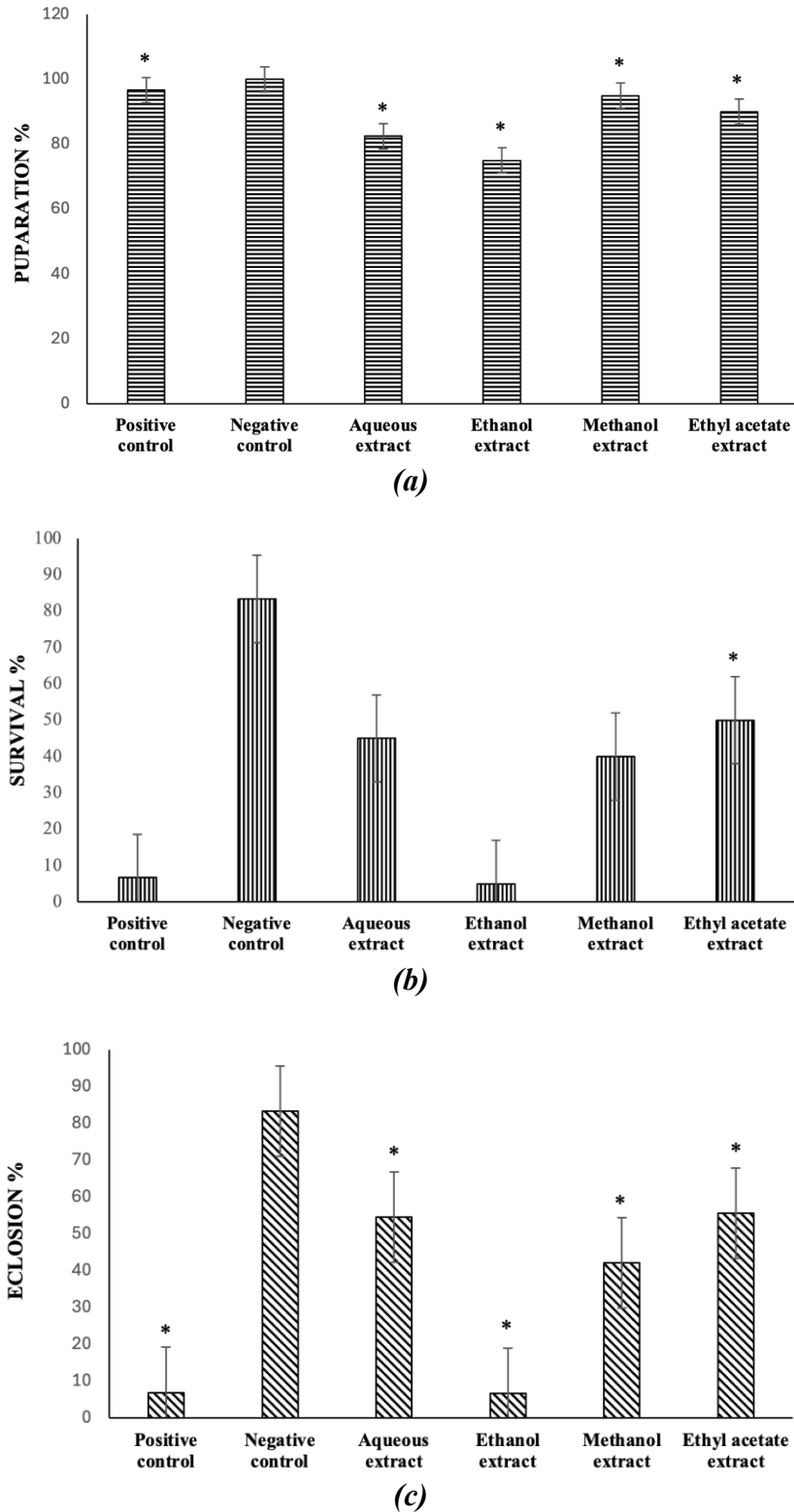


Figure 4. Developmental toxicity test results showing pupuration % (a), survival % (b) and eclosion % (c) following exposure with aqueous, ethanol, methanol and ethyl acetate extracts of *S. syriaca* (*, indicates statistical significance compared to the negative control ($p < 0.05$)).

3.2. *In vivo* antioxidant activity test results

Plants are known to have important antioxidant molecules essential for healthy plant functions, adaptation to environment and delivering beneficial properties for human health [52]. The antioxidant capacity of the plants is routinely addressed in academic studies by *in vitro* tests. However, the actual presence of antioxidants at tissue levels depends on absorption, distribution, metabolism and excretion. In fact, the measurements based on *in vivo* antioxidant assays are heavily impacted by the physiological conditions of an organism [53]. Therefore, in this study *in vivo* antioxidant activities were tested as another tool for toxicity testing and the results are given in Figure 5, 6 and Table 1. According to the results given in Figure 5 and 6, all extracts of *S. suffruticosa* demonstrated high *in vivo* antioxidant capacities ($\geq 80\%$ activity) by ABTS test, while the methanolic and ethanolic extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis* and *S. syriaca* showed high antioxidant activities as a result of the DPPH test ($>50\text{--}70\%$ activity, $p < 0.05$, Figure 5). ABTS test results also showed that all of the plant extracts showed antioxidant activities equivalent to or higher than the negative control (Figure 6). Moreover, FRAP results showed that all of the plants caused high antioxidant capacities compared to negative control ($p < 0.05$, Table 1). All extracts of *S. candidissima* subsp. *candidissima* showed $0.0122 - 0.0234\ \mu\text{g TE/g}$ antioxidant capacity, *S. multicaulis* showed $0.0025\text{--}0.0060\ \mu\text{g TE/g}$ antioxidant capacity, *S. suffruticosa* showed $0.0049\text{--}0.0061\ \mu\text{g TE/g}$ antioxidant capacity and *S. syriaca* showed $0.0045\text{--}0.0143\ \mu\text{g TE/g}$ antioxidant capacity. In conclusion, the Trolox equivalent antioxidant capacities (TEAC) reported here for *S. multicaulis* ($0.0025\text{--}0.0060\ \mu\text{g TE/g}$) and *S. suffruticosa* ($0.0049\text{--}0.0061\ \mu\text{g TE/g}$) indicate strong antioxidant potential. TEAC is a widely used metric for quantifying antioxidant activity, where results are expressed in terms of Trolox equivalents—Trolox being a water-soluble analog of vitamin E and a standard reference antioxidant. A higher TEAC value reflects a greater ability of the extract to neutralize free radicals, effectively mirroring the antioxidant strength of an equivalent amount of Trolox [54]. Notably, *S. multicaulis* and *S. suffruticosa* displayed the lowest TEAC values among the tested species, meaning they required the least amount of extract to exert an antioxidant effect equivalent to Trolox, and thus possess the highest antioxidant potency. In contrast, *S. syriaca* and *S. candidissima* subsp. *candidissima* exhibited higher TEAC values, indicating lower antioxidant strength in comparison. Comparative surveys of Turkish medicinal plants have shown TEAC values ranging from 17.6 to $1472\ \mu\text{mol}$ Trolox equivalents per gram dry weight, and EC_{50} values from approximately 0.174 to $42\ \text{mg/g}$ dry matter—placing the most active *Salvia* extracts from this study at the more potent end of this spectrum [55]. Although *in vivo* studies rarely report antioxidant activity using identical units, experimental findings such as the amelioration of scopolamine-induced cognitive deficits by *S. multicaulis* essential oil in rodent models provide initial evidence of its systemic antioxidant and neuroprotective effects [56]. Taken together, these results suggest that *S. multicaulis* and *S. suffruticosa* in particular merit further investigation in *in vivo* systems to validate their antioxidant efficacy under physiological conditions.

Different antioxidant capacity assays such as DPPH, ABTS, and FRAP often yield different results when applied to the same sample. These discrepancies primarily arise from the distinct chemical principles underlying each method. DPPH and ABTS assays evaluate radical scavenging activity, whereas the FRAP assay measures the reducing power based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Moreover, while DPPH and ABTS involve both single electron transfer (SET) and, to a lesser extent, hydrogen atom transfer (HAT) mechanisms, the FRAP assay operates exclusively via the

SET mechanism [57]. Environmental factors such as pH, solvent polarity, and the solubility of antioxidant compounds also significantly affect their reactivity with assay reagents [58]. The low correlation frequently observed between these assays—particularly the deviation of DPPH from others—has been attributed to their differing sensitivities to various classes of antioxidant molecules. Therefore, to obtain a more accurate and comprehensive evaluation of antioxidant capacity, it is recommended to use multiple complementary assays [59].

To conclude the results of the *in vivo* antioxidant activity, it is demonstrated in this study that the studied plant extracts didn't harm the physiological antioxidant system (balance) of the whole organism. There are some observations with other plant species in literature, but no studies have been published for *in vivo* antioxidant activities of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* plant extracts in *D. melanogaster* previously to compare those results. On the other hand, some studies investigated the *in vivo* applications of *Salvia* species using different model organisms by investigating the plasma antioxidant levels. In a separate study, researchers examined the impact of methanolic leaf extracts of *Salvia officinalis* on antioxidant enzymes—including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)—as well as on plasma lipid parameters such as triglycerides (TG), total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) in Wistar rats with Alloxan-induced diabetes. Compared to untreated diabetic rats, those exposed to the sage extract showed a 60% and 33% increase in SOD activity and reductions in TG and LDL levels by 40% and 30%, respectively. Metformin demonstrated only moderate antioxidant and lipid-lowering effects. These findings suggest that *S. officinalis* may offer therapeutic potential for diabetes management through its significant antioxidant and hypolipidemic actions [60].

The researchers also found that the aqueous extract of *Choerospondias axillaris* fruit significantly attenuated oxidative damage in D-galactose-induced aging mice, demonstrating notable *in vivo* antioxidant activity suggesting its potential as a natural antioxidant agent in managing oxidative stress-related conditions [61]. In another study, The ethanolic extract of *Swertia chirayita* significantly restored antioxidant defenses—elevating superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels while reducing malondialdehyde (MDA)—in liver and kidney tissues of carbon tetrachloride (CCl₄)-intoxicated mice, demonstrating potent *in vivo* antioxidant activity [62].

The cold aqueous leaf extract of *Mangifera indica* also significantly enhanced survival rates (100% at 2.5 mg/10 g and 85% at 5 mg/10 g diet) and boosted antioxidant enzyme activity—elevating catalase, glutathione-S-transferase, and total thiol levels—in *D. melanogaster*, indicating potent *in vivo* antioxidant protective effects [63].

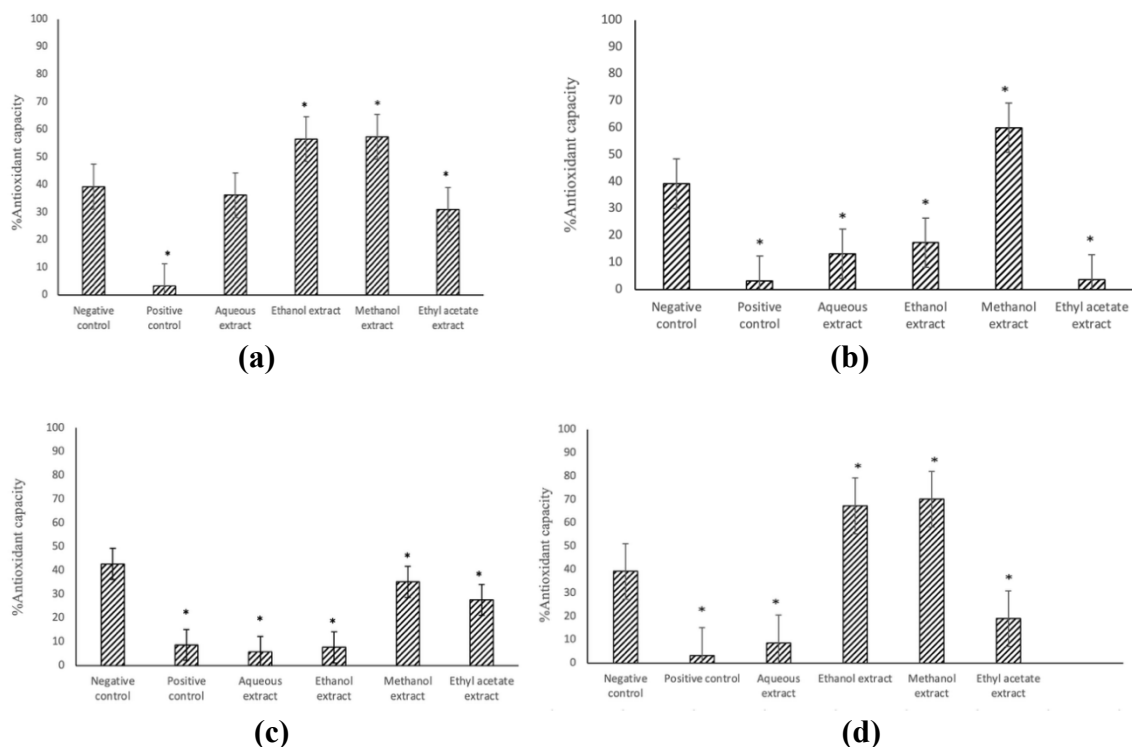


Figure 5. In vivo DPPH test results of *S. candidissima* subsp. *candidissima* (a), *S. multicaulis* (b), *S. suffruticosa* (c) and *S. syriaca* (d) (*, indicates statistical significance compared to the negative control ($p < 0.05$)).

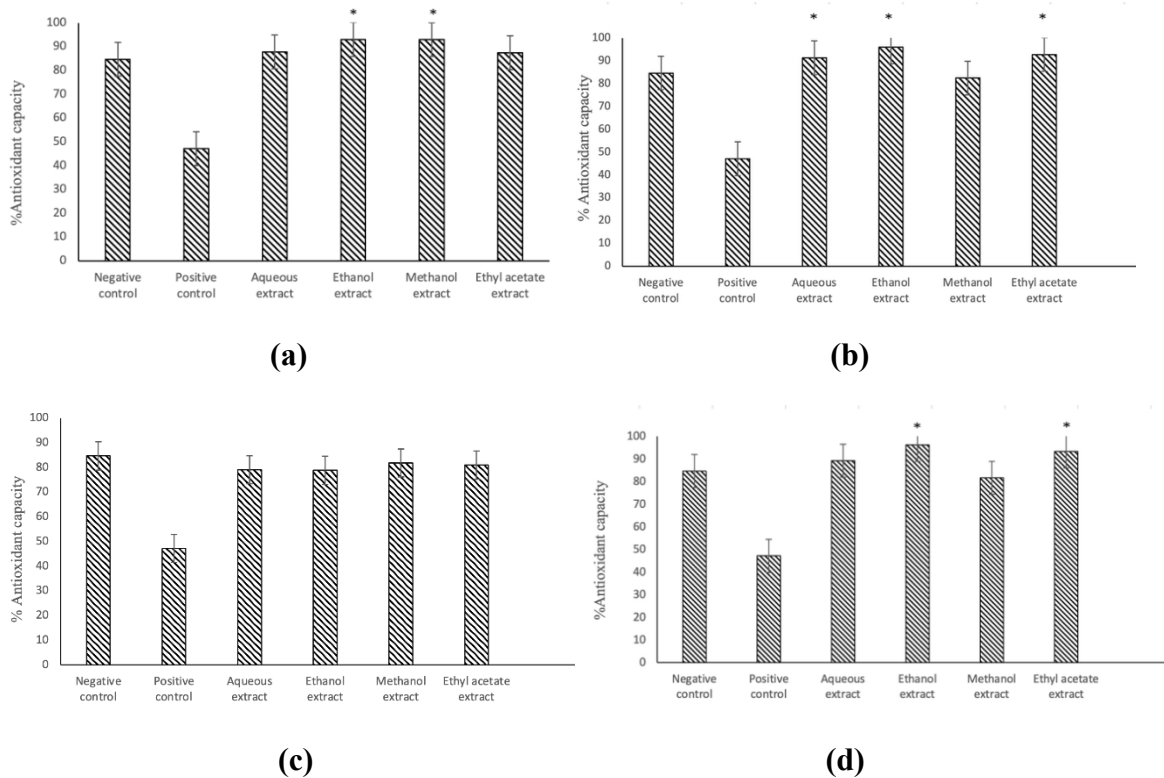


Figure 6. In vivo ABTS test results of *S. candidissima* subsp. *candidissima* (a), *S. multicaulis* (b), *S. suffruticosa* (c) and *S. syriaca* (d) (*, indicates statistical significance compared to the negative control ($p < 0.05$)).

Table 1. In vivo FRAP test results (Mean $\pm$ SE, * $p < 0.05$ compared to the H_2O_2 group, TE: Trolox equivalent)

Plant	Extract Type	FRAP (μ g TE/g)
H_2O_2 (6.5 μ g/mL)		0.0078 $\pm$ 0.0001
<i>S. candidissima</i> subsp. <i>candidissima</i>	Aqueous	0.0204 $\pm$ 0.0001*
	Ethanollic	0.0147 $\pm$ 0.0003*
	Methanolic	0.0122 $\pm$ 0.0001*
	Ethyl Acetate	0.0234 $\pm$ 0.0003*
<i>S. multicaulis</i>	Aqueous	0.0042 $\pm$ 0.000007*
	Ethanollic	0.0034 $\pm$ 0.000003*
	Methanolic	0.0025 $\pm$ 0.000002*
	Ethyl Acetate	0.0060 $\pm$ 0.000008*
<i>S. suffruticosa</i>	Aqueous	0.0049 $\pm$ 0.000009*
	Ethanollic	0.0051 $\pm$ 0.0003*
	Methanolic	0.0061 $\pm$ 0.0002*
	Ethyl Acetate	0.0078 $\pm$ 0.0006
<i>S. syriaca</i>	Aqueous	0.0045 $\pm$ 0.0002*
	Ethanollic	0.0143 $\pm$ 0.0007*
	Methanolic	0.0133 $\pm$ 0.0001*
	Ethyl Acetate	0.0083 $\pm$ 0.0001*

4. Conclusion

Many active compounds of *Salvia* species are known to have the potential to be developed as novel drugs [64]. Therefore, the toxicological profile of every endemic species should be studied in detail. In this study, different extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis*, *S. suffruticosa* and *S. syriaca* were studied to investigate the developmental toxicity and *in vivo* antioxidant effects on *D. melanogaster*. The developmental toxicity test results showed that *S. suffruticosa* extracts gave much higher values than the positive control among the other plant extracts and weren't toxic. The results indicated that none of the extracts of *S. suffruticosa* except the methanolic extract affected the pupuration and survival rates ($p > 0.05$), while other plant extracts negatively influenced the normal developmental processes. All extracts of *S. suffruticosa* demonstrated high *in vivo* antioxidant capacities (≥ 80 % activity) according to the ABTS test, while methanolic and ethanollic extracts of *S. candidissima* subsp. *candidissima*, *S. multicaulis* and *S. syriaca* showed high antioxidant activities according to DPPH test (>50 -70 % activity). FRAP test also showed that the Trolox equivalent antioxidant capacities reported here for *S. multicaulis* (0.0025–0.0060 μ g TE/g) and *S. suffruticosa* (0.0049–0.0061 μ g TE/g) indicate strong antioxidant potential. Differences in antioxidant activity among plant species are determined by multiple interacting factors. First, the quantitative content of antioxidant compounds (total phenolics, flavonoids, tannins) varies and species, genotype, and even ecogeographic origin contribute significantly to this variation [65]. Second, qualitative differences like the molecular structure of phenolic compounds (degree of hydroxylation, conjugation, glycosylation) greatly affect radical scavenging, metal-chelating, or enzyme-inhibitory potential. Third, extraction method, solvent polarity, plant part, maturation stage and environmental factors (light,

altitude, soil nutrients) also influence both the yield and profile of antioxidant constituents [66], [67]. Finally, in many species there is a trade-off (or complementary action) between antioxidant enzyme systems (e.g. superoxide dismutase, catalase, glutathione-system) and non-enzymatic compounds and the relative contribution varies across species [68], [69]. To conclude the results of the *in vivo* antioxidant activity, it is demonstrated in this study that the studied plant extracts didn't harm the physiological antioxidant system (balance) of the whole organism and the studied *Salvia* species have strong potential for the development of effective natural antioxidant supplements.

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