

Research Article

Antioxidant Activity and Reactive Oxygen Species (ROS) Scavenging Mechanism of *Eriodictyon californicum*, an Edible Herb of North America

Allie Richards and Savita Chaurasia 

Department of Chemistry, Bellarmine University, Louisville, KY 40205, USA

Correspondence should be addressed to Savita Chaurasia; schaurasia@bellarmine.edu

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Modern life associated with processed food, exposure to a wide range of pollutants, physical inactivity, unprecedented time, excessive involvement of technology in our lives, and so on give rise to oxidative stress which threatens our physical and mental health. Because of rising healthcare costs and mistrust of conventional medicine, mankind is looking for alternatives to over-the-counter medications and prescription drugs, which is driving the demand for nutraceuticals across the globe. Antioxidant-rich plant-based natural products could be an important source of nutraceuticals to boost endogenous antioxidant levels. In this research, we uncovered the antioxidant potential of *Eriodictyon californicum*, an herb used by Native Americans for centuries to treat a wide range of ailments. The ethanolic leaf extract of *E. californicum* was studied for its phytochemical constituents, antioxidant potential, and free radical scavenging activity. Phytochemical analysis revealed the presence of flavonoids, saponins, phenols, tannins, terpenoids, cardiac glycosides, and steroids. The abundance of total phenol ($78.58 \pm 0.016 \mu\text{g GAE/mg}$) and flavonoid content ($6.76 \pm 0.003 \mu\text{g QE/mg}$) and the strong reducing activity in the FRAP assay indicated the antioxidant potential of the leaves. Free radical scavenging activity was confirmed by a dose-dependent response on the DPPH assay ($\text{IC}_{50} 0.071 \text{ mg/mL}$). Since reactive oxygen species are the main culprits for causing oxidative stress in our body, studying the effect of plant extracts on superoxide, hydrogen peroxide, and hydroxyl radicals found that it has the ability to neutralize all three. IC_{50} was found to be 0.083 mg/mL for superoxide radicals, 0.033 mg/mL for hydrogen peroxide, and 0.080 mg/mL for hydroxyl radicals. Herein, we report that *E. californicum* is a potential source of bioactive components with strong radical scavenging activity. This holy herb could serve as a healing agent against oxidative stress and holds promise for the development of nutraceuticals.

1. Introduction

We make every effort to eat a balanced diet, but we do not get all the micronutrients needed to maintain good health. Therefore, we keep looking for supplements and nutrients to maintain our health, and plant-derived nutraceuticals appear to be a good choice because of their perceived safety and potential nutritional and therapeutic effects. Nutraceuticals are naturally derived bioactive compounds found in foods, dietary supplements, and herbal products, and have health-promoting, as well as disease-preventive and medicinal properties [1–3]. Plant-based nutraceuticals are in increasing demand due to their rich bioactive profile,

and their multiple and pleiotropic activity without side effects [4, 5]. In most countries, including the United States, nutraceuticals are included in the dietary supplement list and are not subject to laws and regulations for the safety standards of allopathic medicine [6, 7]. The use of herbal extracts in the nutraceuticals market is projected to reach US\$5.8 billion by 2031 [8].

Antioxidant-rich natural products represent a growing class of nutraceuticals [9, 10]. Antioxidants protect against oxidative stress by interrupting the propagation of free radicals or inhibiting the formation of free radicals. Oxidative stress has been implicated in the genesis and progression of various human diseases and conditions,

including diabetes, arthritis, cancer, atherosclerosis, heart attack, Alzheimer's disease, Parkinson's, and many more [11–14]. Clinical research reveals that consuming foods rich in antioxidants and polyphenols boosts the antioxidant capacity of our body and reduces the damage caused by oxidative stress [15–19]. Thus, including plant-based antioxidant-rich nutrients in our diet can help prevent us from various chronic diseases.

Taking a step in this direction, we aimed to study the bioactive components and antioxidant potential of the plant *Eriodictyon californicum* (Hook. & Arn.) Torr., which is an evergreen shrub in the family Boraginaceae and is native to California, Oregon, and northern Mexico. It is commonly known as California Yerba Santa meaning sacred herb [20] and has been used for centuries by Native American and Spanish settlers for various ailments including cold, cough, asthma, fever, sores, and stomach problems. It was also used as a poultice for the management of bruises, wounds, and rheumatic pain [21–23]. Yerba Santa appears as a flavoring agent in the FDA GRAS list (U.S. Food and Drug Administration; Generally Recognized As Safe) [24]. Products made from Yerba Santa are used in food applications and pharmaceuticals as a taste modifier. The phytochemical analysis illustrates the presence of various bitter masking flavonoids [25, 26]. The bioactive flavonoids present in the plant have also shown cancer chemoprevention and anti-tumor properties [27, 28]. Recently, flavanone sterubin has been identified as an active compound with potent neuro-protective, anti-inflammatory, and iron chelating activities [29, 30]. We first revealed the antioxidant potential of *Eriodictyon californicum* and presented our preliminary research findings at an international conference [31]. The current study emphasizes the antioxidant potential of Yerba Santa to help prevent oxidative stress. For this, different groups of phytochemicals present in plant extracts have been analyzed by qualitative tests, and the total phenolic content and flavonoids have been quantified. Antioxidant potential was first assessed by FRAP assay and DPPH assay and then its effect on oxygen-borne free radicals has been studied to extract the exact mechanism of action as an antioxidant. The findings of this study provide a scientific basis for the traditional claims of this plant, support its practical use in the food industry, and pave the way for the development of nutraceuticals with antioxidant potential.

2. Materials and Methods

2.1. Plant Material and Chemicals. In traditional American health practices, the use of the leaves of *E. californicum* has been described to treat a variety of ailments [21–23]. The leaves are still used as a food flavoring and as an additive to some medicines to mask the bitter taste of other ingredients [24]. Therefore, the leaves of the plant have been used in this study. The dried whole leaves of *Eriodictyon californicum* were purchased from Monterey Bay Spice Company, Watsonville, CA, USA, Lot No. 19G212-287. All chemicals used in the study were analytical grade and were obtained from Sigma-Aldrich, Inc., St. Louis, MO, USA.

2.2. Extract Preparation. The methanol extract was prepared by the Soxhlet extraction method [32]. Briefly, 50 g dried leaves of *Eriodictyon californicum* were cut, coarsely powdered, and extracted using Soxhlet with 500 mL of 95% ethanol at 60–80°C for 12 hours. The extract was concentrated by rotovapping, and the residue was dried by desiccating. The percent yield of the *Eriodictyon californicum* leaf extract (ECE) was 35.07%. The extract was diluted to a known concentration (w/v) by suspending it in the drug vehicle (Tween 20: water, 1 : 9).

2.3. Qualitative and Quantitative Analysis of Phytochemicals

2.3.1. Qualitative Assay of Phytochemicals. To identify different classes of active chemical constituents, a qualitative analysis of ECE was carried out using standard methods described by Ajayi et al. [33] with slight modifications [34]. 10 mg/mL stock solution of ECE was used for all tests except carotenoids.

Tannins: 0.5 mL of ECE was combined with 1 mL of deionized water (DIW). 3 drops of 5% ferric chloride were added to the solution. The appearance of a blue-green or blue-black color indicated the presence of tannins.

Phlobatannins: 0.5 mL of ECE was boiled with 1% HCl. The formation of a red precipitate indicated the presence of phlobatannins.

Flavonoids: 3 drops of 20% NaOH were combined with 0.5 mL of ECE. The presence of an intense yellow color that disappeared with the addition of dilute acid indicated the presence of flavonoids.

Saponins: 0.5 mL of ECE was combined with 0.5 mL of DIW and shaken vigorously. After 10 minutes, the frothing solution was mixed with 3 drops of olive oil and shaken vigorously again. The presence of a stable persistent froth prior to the addition of olive oil and an emulsion after the addition of olive oil indicated the presence of saponins.

Terpenoids: 0.5 mL of ECE was combined with 2.0 mL of chloroform and 3.0 mL of concentrated H₂SO₄ to form layers. The presence of a red-brown color at the interface indicated the presence of terpenoids.

Cardiac Glycosides: 0.5 mL ECE was combined with 2.0 mL of glacial acetic acid and one drop of ferric chloride solution. 1.0 mL of concentrated H₂SO₄ was carefully added along the sides of the test tube. The presence of a brown ring at the interface indicates the presence of cardiac glycosides.

Free Anthraquinones: 0.5 mL ECE was combined with 1 mL of chloroform and mixed vigorously for 5 minutes. The solution was filtered and the filtrate was shaken with an equal volume of 10% ammonia. The presence of a pink, red, or violet color indicated the presence of anthraquinones.

Carotenoids: 10 mL of chloroform was combined with 1.0 g of concentrated ECE and mixed vigorously. The mixture was filtered and concentrated sulfuric acid was added to the filtrate. The presence of blue color at the interface of the aqueous and organic layer indicated the presence of carotenoids.

Alkaloids: 2.0 mL of Wagner's reagent (1.27 g of iodine and 2.0 g of potassium iodide in 100 mL distilled water) was

added to 0.5 mL ECE. The presence of a red or brown precipitate indicated the presence of alkaloids.

Steroids: 0.5 mL of ECE was stirred with 2.0 mL of chloroform and 2.0 mL of concentrated H₂SO₄ was added to the mixture. The presence of red color in the chloroform layer indicated the presence of steroids.

2.3.2. Quantitation of Total Phenolic and Flavonoid Content.

The total phenolic content of ECE was determined using the Folin–Ciocalteu (FC) method [35]. Briefly, 20 μ L of ECE (10 mg/mL) was diluted to 5.0 mL with distilled water, and 0.5 mL of 1 : 1 FC reagent was added to this solution. After 5 minutes of incubation, 5.0 mL of 7% sodium carbonate was added and the mixture was incubated for 30 minutes at room temperature and then the absorbance was measured at 765 nm. Gallic acid was used as a standard and the regression equation derived from the gallic acid calibration curve was used to calculate the total phenolic content. The results were expressed as μ g of gallic acid equivalents per milligram of plant material (μ g GAE/mg plant material).

The total flavonoid content of ECE was determined by the modified aluminum chloride colorimetric method [36]. Briefly, 20 μ L of ECE (10 mg/mL) was diluted to 5.0 mL with distilled water and then 0.1 mL of 10% AlCl₃ and 0.1 mL of 1 M potassium acetate were added. This mixture was incubated at 37°C for 40 minutes and the absorbance was measured at 415 nm. Quercetin was used as a standard, and the flavonoid concentration in ECE was calculated from the regression equation derived from the quercetin calibration curve. The results were expressed as μ g of quercetin equivalents per milligram of plant material (μ g QE/mg plant material).

2.4. Determination of Antioxidant Potential. The antioxidant potential of ECE was determined by measuring the ability of ECE to donate an electron to reduce Fe³⁺ to Fe²⁺ in the FRAP assay or donate a hydrogen atom to stabilize a synthetic-free radical in the DPPH assay.

2.4.1. Ferric Reducing Antioxidant Power (FRAP) Assay.

The total antioxidant capacity of plant extract was determined by FRAP assay [37]. 0.5 mL of ECE with different concentrations (0.1 to 1.0 mg/mL) was mixed with 2.0 mL of phosphate buffer (50 mM, pH 7.0) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes followed by the addition of 2.5 mL of 10% TCA. The reaction mixture was centrifuged at 3000 \times g for 10 minutes. 1.25 mL supernatant from each sample was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% ferric chloride. Absorbances were measured at 700 nm. Ascorbic acid (AA) was used as a standard.

2.4.2. DPPH Assay. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable synthetic-free radical widely used to investigate the free radical scavenging activity of natural products or synthetic compounds. Preliminary screening of the free radical scavenging activity of ECE was carried out by the

method developed by Blois [38] with slight modifications. 0.3 mM solution of DPPH was prepared in methanol and incubated in the dark for 2 hours. 1.0 mL of ECE and AA with different concentrations (0.025 to 0.50 mg/mL) were mixed with 0.5 mL of DPPH. The reaction mixture was vortexed and incubated in the dark at room temperature for 30 minutes, and the absorbance was read at 517 nm.

2.5. Free Radical Scavenging Assay. The free radical scavenging activity of ECE was studied with oxygen-derived free radicals to elucidate the mechanism of action.

2.5.1. Superoxide Radical Scavenging Assay. Superoxide anion free radical scavenging activity was measured by the pyrogallol autoxidation method [39]. 0.100 mL of ECE and AA at different concentrations (0.010 to 0.100 mg/mL) were thoroughly mixed with 0.880 mL of tris-HCl buffer (0.05 M, pH 7.4) and 0.02 mL of pyrogallol (60 mM) and the absorbance was measured at 325 nm every 30 seconds for three minutes. The change in absorbance (ΔA) in 3 minutes was used to calculate the % scavenging activity.

2.5.2. Hydrogen Peroxide Decomposition Assay. The ability of ECE to decompose hydrogen peroxide was studied by the method described by Aebi [40] with a slight modification. 20 mM hydrogen peroxide solution was prepared in 50 mM phosphate buffer (pH 7.4). 0.4 mL of ECE and AA with varying concentrations (0.001 to 0.050 mg/mL) were mixed with 0.6 mL of hydrogen peroxide solution. After incubation for 10 minutes at room temperature, the absorbance was measured against the blank at 240 nm. The blank consisted of phosphate buffer and test samples without hydrogen peroxide.

2.5.3. Hydroxyl Radical Scavenging Assay. The hydroxyl radical scavenging activity of ECE was determined by a 2-deoxyribose degradation assay developed by Gutteridge and Halliwell [41]. In brief, the reaction mixture contained 0.01 mL FeCl₃ (10 mM), 0.1 mL EDTA (1 mM), 0.1 mL H₂O₂ (10 mM), 0.36 mL deoxyribose (10 mM), 1.0 mL ECE of various concentrations (0.05 to 0.75 mg/mL), and 0.3 mL phosphate buffer (0.05 M, pH 7.4). The reaction was initiated by adding 0.1 mL ascorbic acid (1 mM). After incubation of the reaction mixture at 37°C for 1 hour, 1.0 mL of the test sample was added to 1.0 mL of TCA (10%) and was mixed with 1.0 mL of TBA (10%) and heated in a boiling water bath for 30 minutes. The absorbance was read at 532 nm and the percent inhibitory activity was calculated. Mannitol was used as the standard.

The percentage of radical scavenging activity was calculated using the following equation:

$$\% \text{ free radical scavenging activity} = \left[\frac{(Ac - As)}{Ac} \right] \times 100, \quad (1)$$

where Ac is the absorbance of the control, and As is the absorbance of plant extract/standard.

2.6. *Statistical Analysis.* Statistical analysis was performed using the Microsoft Excel program (2019). Experimental results were expressed as a mean \pm standard deviation of three independent experiments. The level of significance has been evaluated by using the Student's *t*-test. The *P* value < 0.05 was considered statistically significant. The IC_{50} was determined by linear regression analysis.

3. Results and Discussion

3.1. Qualitative and Quantitative Assay of Phytochemicals

3.1.1. *Qualitative Assay of Phytochemicals.* Phytochemical screening of ECE revealed the presence of tannins, phlobatannins, phenols, flavonoids, saponins, terpenoids, cardiac glycosides, and steroids, while free anthraquinones, carotenoids, and alkaloids were absent (Table 1). The concentration of phytochemicals was determined visually by the intensity of the color. The absence of phytochemicals is indicated by (–), the moderate presence is indicated by (+), and (++) indicates the presence of phytochemicals in high amounts. The presence of these bioactive phytoconstituents substantiates the health-promoting and disease-prevention properties of yerba santa described in the literature [21–23].

3.1.2. *Total Phenolic and Flavonoid Content.* Phenolic compounds and flavonoids are well known for their antioxidant properties. These secondary metabolites neutralize free radicals by donating hydrogen and terminating the chain for the generation of new radicals [42]. This study confirmed the presence of polyphenols in the leaves of yerba santa. The total phenolic content of ECE was found to be $78.68 \pm 0.016 \mu\text{g GAE/mg}$ and flavonoid content was determined to be $6.76 \pm 0.003 \mu\text{g QE/mg}$. The presence of these phytochemicals in ECE points to the antioxidant potential of the plant. Further investigation was carried out to confirm its antioxidant properties and to provide a scientific explanation for its action as an antioxidant.

3.2. Determination of Antioxidant Potential

3.2.1. *Ferric Reducing Antioxidant Power (FRAP) Assay.* Antioxidants neutralize free radicals by donating an electron. The FRAP assay is used to determine the electron donating activity of substances by reducing potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric–ferrous complex with an absorption maximum of 700 nm. The higher the reducing power, the greater the absorbance of the reaction mixture [43]. The increase in absorbance at 700 nm indicated the reducing power of ECE, which was comparable to that of standard AA (Figure 1). A concentration-dependent increase in absorbance was observed with both ECE ($R^2 = 0.9886$) and AA ($R^2 = 0.972$). The value of R^2 demonstrated a strong positive correlation between the concentrations of ECE and AA and their reducing potential. This observation reflects the ECE's ability to donate

TABLE 1: Qualitative phytochemical analysis of ECE.

Phytochemical	Availability
Tannins	++
Phlobatannins	+
Phenols	+
Flavonoids	++
Saponins	++
Terpenoids	++
Cardiac glycosides	+
Free anthraquinones	–
Carotenoids	–
Alkaloids	–
Steroids	++

(++) present in high amount; (+) moderate amount; (–) completely absent.

electrons, which may be a major contributor to neutralizing free radicals.

3.2.2. *DPPH Radical Scavenging Activity.* Hydrogen atom transfer (HAT) is an important mechanism in detoxifying free radicals [44]. The ability of *E. californicum* to transfer hydrogen atoms to neutralize free radicals was studied on the DPPH assay. The DPPH is a stable synthetic radical at room temperature that produces a violet solution in ethanol. It is reduced through the addition of hydrogen, causing a color change from violet to clear [45]. The results in Table 2 demonstrate the concentration-dependent DPPH radical scavenging activity of ECE and standard AA. At a concentration of 0.500 mg/mL, ECE and AA exhibited 80.83 ± 0.17 and $99.98 \pm 0.87\%$ DPPH radical scavenging, respectively. The IC_{50} for AA was found to be 0.018 mg/mL and for ECE it was 0.071 mg/mL. This observation revealed the H-atom-donating ability of ECE.

3.3. *Free Radical Scavenging Activity.* Reactive oxygen species (ROS), namely, superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO^{\bullet}) are continuously produced in our body during normal physiological processes when molecular oxygen undergoes univalent reduction. High levels of ROS are known to promote cell damage and death and have been implicated in the etiology of various chronic and degenerative diseases [46]. Thus to explore the ability of ECE to help us protect against ROS-induced oxidative stress, ROS scavenging activity was investigated by studying the effect of ECE on all three radicals.

3.3.1. *Superoxide Radical Scavenging Activity.* Superoxide radicals were generated by the autooxidation of pyrogallol. ECE showed a dose-dependent scavenging activity that was comparable to standard ascorbic acid (Table 3). At a dose of 0.1 mg/mL, ECE exhibited $57.37 \pm 5.01\%$ inhibition of superoxide radicals, which was higher than the value obtained with standard AA $51.08 \pm 3.87\%$. This activity could not be studied at higher concentrations because the color of the plant extract interfered with the absorbance in the UV region. However, the results obtained at the concentrations under study clearly indicated that ECE has

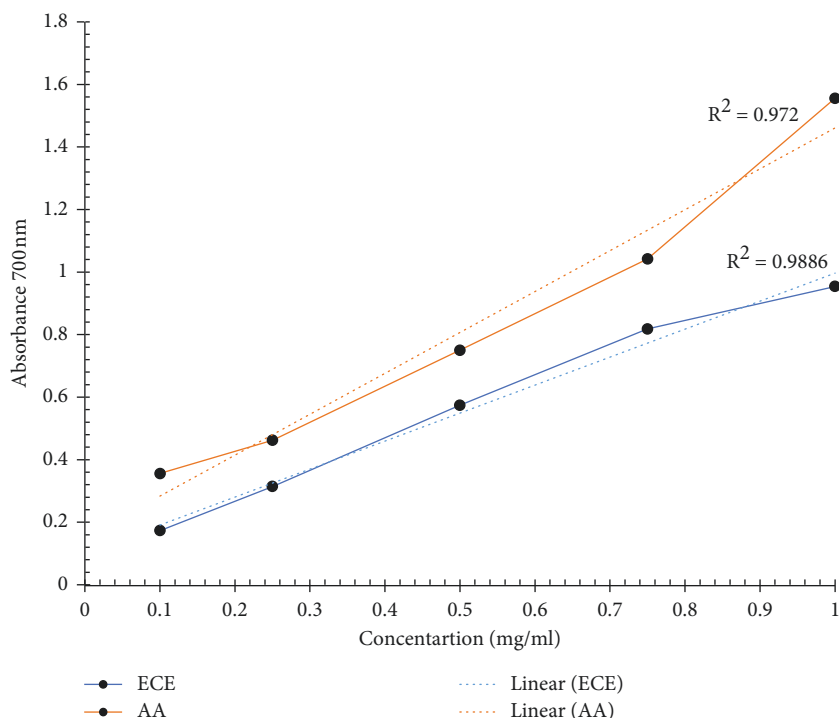


FIGURE 1: Ferric reducing the activity of *E. californicum* extract (ECE) and ascorbic acid (AA). Each data point is expressed as mean \pm standard deviation ($n = 3$); $P < 0.05$.

TABLE 2: DPPH radical scavenging activity of *E. californicum* extract (ECE) and ascorbic acid (AA).

Concentration (mg/mL)	% DPPH radical scavenging	
	ECE	AA
0.025	07.98 \pm 2.35	20.07 \pm 1.09
0.050	33.60 \pm 1.56	46.86 \pm 1.28
0.100	59.56 \pm 1.21	72.07 \pm 1.01
0.200	62.08 \pm 1.41	84.45 \pm 0.95
0.500	80.83 \pm 0.17	99.98 \pm 0.87
IC ₅₀	0.071 mg/mL	0.018 mg/mL

Each value is expressed as mean \pm standard deviation ($n = 3$); $P < 0.05$.

TABLE 3: Superoxide radical scavenging activity of *E. californicum* extract (ECE) and ascorbic acid (AA).

Concentration (mg/mL)	% Superoxide radical scavenging	
	ECE	AA
0.010	12.18 \pm 2.91	11.82 \pm 1.06
0.025	18.11 \pm 2.22	16.71 \pm 1.73
0.050	30.29 \pm 2.91	28.86 \pm 1.42
0.075	46.40 \pm 1.34	42.42 \pm 3.13
0.100	57.37 \pm 5.01	51.30 \pm 2.99
IC ₅₀	0.083 mg/mL	0.096 mg/mL

Each value is expressed as mean \pm standard deviation ($n = 3$); $P < 0.05$.

the ability to quench superoxide radicals and thus exhibits superoxide dismutase-like activity that can act as a defense against ROS.

TABLE 4: Hydrogen peroxide decomposition activity of *E. californicum* extract (ECE) and ascorbic acid (AA).

Concentration (mg/mL)	% H ₂ O ₂ decomposition	
	ECE	AA
0.001	09.94 \pm 1.64	13.66 \pm 0.92
0.005	20.73 \pm 1.11	26.55 \pm 0.54
0.010	34.18 \pm 1.40	42.51 \pm 0.63
0.020	41.76 \pm 1.13	86.69 \pm 1.77
0.050	60.45 \pm 1.42	103.67 \pm 3.51
IC ₅₀	0.033 mg/mL	0.012 mg/mL

Each value is expressed as mean \pm standard deviation ($n = 3$); $P < 0.05$.

3.3.2. Hydrogen Peroxide Decomposition Activity. Hydrogen peroxide is a moderately reactive ROS that is formed by the dismutation of superoxide radicals either nonenzymatically under low pH conditions or mostly by inflammatory cells and triggers oxidative stress. The effect of ECE on hydrogen peroxide was investigated, and AA served as a positive control. The results in Table 4 are evidence that ECE effectively decomposed hydrogen peroxide in a concentration-dependent manner. 50% decomposition was obtained at a concentration of 0.033 mg/mL and 0.012 mg/mL for ECE and AA, respectively. The decomposition of hydrogen peroxide can be attributed to the presence of phenolic groups that can donate electrons to hydrogen peroxide, deactivating it to H₂O.

3.3.3. Hydroxyl Radical Scavenging Activity. Hydroxyl radicals are considered key players in oxidative damage due

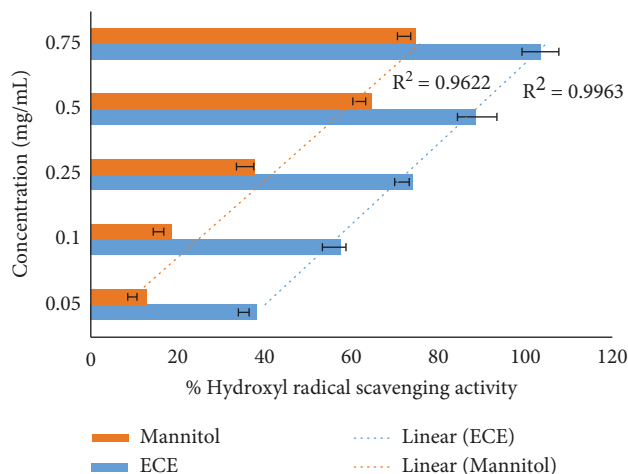


FIGURE 2: Hydroxyl radical scavenging activity of *E. californicum* extract (ECE) and mannitol. Each value is expressed as mean \pm standard deviation ($n = 3$); $P < 0.05$.

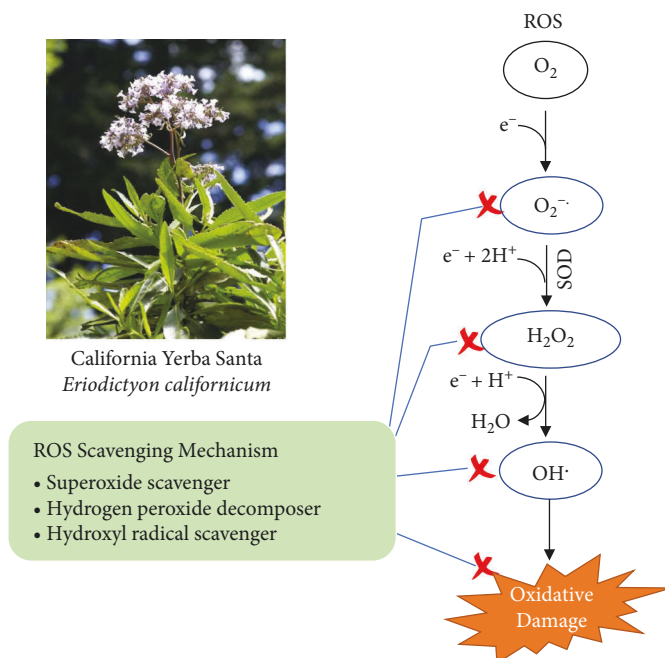


FIGURE 3: Role of *E. californicum* in the prevention of ROS-induced oxidative stress. ROS are highly reactive chemicals formed from O₂. The univalent reduction of molecular oxygen (O₂) produces superoxide radicals (O₂^{•-}), hydrogen peroxide (H₂O₂), and hydroxyl radicals (HO[•]). ECE scavenged superoxide and the hydroxyl radical and decomposed hydrogen peroxide.

to their extremely high reactivity and short half-life. Our body has not developed any enzymes to detoxify this radical, so its excessive accumulation induces cell death [47]. The ability of ECE to scavenge hydroxyl radicals was determined by inhibiting the decomposition of deoxyribose by hydroxyl radicals generated from the Fenton reaction (Fe³⁺-ascorbate-EDTA-H₂O₂). Mannitol, a well-known hydroxyl radical scavenger, was used as a standard. Both ECE and mannitol exhibited a significant hydroxyl radical neutralizing potential in a concentration-dependent manner, as evident from the linear trendline and R^2 values (0.9622 for

mannitol and 0.9963 for ECE). ECE was found to be most effective in scavenging hydroxyl radicals compared to its effect on superoxide radicals and hydrogen peroxide. At a concentration of 0.75 mg/mL, it showed 103.45% \pm 7.494 inhibition of hydroxyl radicals (Figure 2). At all tested concentrations, ECE outperformed mannitol in hydroxyl radical trapping.

These results indicate that *Eriodictyon californicum* is an important source of natural antioxidants, which might play a vital role in preventing the progression of oxidative stress by scavenging all three ROS (Figure 3).

4. Conclusions

E. californicum is an edible herb that has been traditionally used by Native Americans for various ailments and is also recognized by the U.S. Food and Drug Administration (FDA) as safe; it is used as a flavoring agent in food applications and as a taste modifier in pharmaceuticals. This study explored the antioxidant activity and ROS scavenging mechanism of this holy herb. The results suggest that the antioxidant activity of the *E. californicum* leaf extract is due to synergy between several mechanisms. The leaves are rich in bioactive compounds that have the ability to neutralize free radicals by single electron transfer (SET) and hydrogen atom transfer (HAT). ECE also scavenged superoxide and hydroxyl radicals and decomposed hydrogen peroxide. Therefore, the leaves of *E. californicum* are a promising source for the development of antioxidant-rich nutraceuticals to help prevent the harmful effects of oxidative stress.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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