

Correlation between Antibacterial Activity and Free-Radical Scavenging: In-Vitro Evaluation of Polar/Non-Polar Extracts from 25 Plants

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Keywords: wild edible plant, natural product, oxidative stress, antioxidant, radical scavenging activity

Abstract:

Objectives: The current study aimed to measure the antioxidant and antibacterial activities of 25 wild Palestinian edible plants, which were subjected to extraction by polar and non-polar solvents. Correlations between free radical scavenging activity and antibacterial activity of the extracts were assessed for both polar and non-polar fractions. **Materials:** Twenty-five wild edible plant species that are frequently consumed by people in Palestine (mainly in a rural area) were examined. Among them, 10 plant species were among those with the highest mean cultural importance values, according to an ethnobotanical survey that was conducted in the West Bank, Palestine, a few years ago. **Method:** The protocol of the DPPH assay for testing free-radical scavenging was utilized for determining EC50 values, while microdilution tests were conducted to determine the 50% inhibitory concentration (IC50) of the extracts for the microorganism *Staphylococcus mutans*. **Results and Discussion:** Eight extracts (non-polar fractions) were found to possess an antibacterial IC50 of less than 20 ppm, such as *Foeniculum vulgare*, *Salvia palaestinafruticose*, *Micromeria fruticose*, *Trigonella foenum-graecum*, *Cichorium pumilum jacq*, *Salvia hierosolymitana boiss*, *Ruta chalepensis*, and *Chrysanthemum coronarium*. The polar fractions possess higher antioxidant activity, while non-polar fraction possess higher antibacterial activity. Looking at all the results together can deceive and lead to the conclusion that there is no correlation between antibacterial activity against *S. mutans* and free radical scavenging (R^2 equals 0.0538). However, in-depth analysis revealed that non-polar plant extracts with an EC50 of free radical scavenging >100 ppm have a four-fold order of enrichment toward more activity against *S. mutans*. These findings are of high importance for screening projects. A four-fold order of enrichment could save plenty of time and many in screening projects. The antibacterial active extracts marked by low-medium free radical scavenging might act through a mechanism of action other than that of highly active, free radical scavenging extracts. **Conclusion:** The screening of antioxidant and antimicrobial activity performed on 25 selected wild plant extracts revealed a satisfactory free radical scavenging and antimicrobial potential that could be of value in the management of oxidative stress. Further studies are recommended to explore novel and highly active natural antibacterial products.

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Article

Correlation between Antibacterial Activity and Free-Radical Scavenging: In-Vitro Evaluation of Polar/Non-Polar Extracts from 25 Plants

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Abstract: Objectives: The current study aimed to measure the antioxidant and antibacterial activities of 25 wild Palestinian edible plants, which were subjected to extraction by polar and non-polar solvents. Correlations between free radical scavenging activity and antibacterial activity of the extracts were assessed for both polar and non-polar fractions. **Materials:** Twenty-five wild edible plant species that are frequently consumed by people in Palestine (mainly in a rural area) were examined. Among them, 10 plant species were among those with the highest mean cultural importance values, according to an ethnobotanical survey that was conducted in the West Bank, Palestine, a few years ago. **Method:** The protocol of the DPPH assay for testing free-radical scavenging was utilized for determining EC₅₀ values, while microdilution tests were conducted to determine the 50% inhibitory concentration (IC₅₀) of the extracts for the microorganism *Staphylococcus mutans*. **Results and Discussion:** Eight extracts (non-polar fractions) were found to possess an antibacterial IC₅₀ of less than 20 ppm, such as *Foeniculum vulgare*, *Salvia palaestinafruticose*, *Micromeria fruticose*, *Trigonella foenum-graecum*, *Cichorium pumilum jacq*, *Salvia hierosolymitana boiss*, *Ruta chalepensis*, and *Chrysanthemum coronarium*. The polar fractions possess higher antioxidant activity, while non-polar fraction possess higher antibacterial activity. Looking at all the results together can deceive and lead to the conclusion that there is no correlation between antibacterial activity against *S. mutans* and free radical scavenging (R^2 equals 0.0538). However, in-depth analysis revealed that non-polar plant extracts with an EC₅₀ of free radical scavenging ≤ 100 ppm have a four-fold order of enrichment toward more activity against *S. mutans*. These findings are of high importance for screening projects. A four-fold order of enrichment could save plenty of time and many in screening projects. The antibacterial active extracts marked by low-medium free radical scavenging might act through a mechanism of action other than that of highly active, free radical scavenging extracts. **Conclusion:** The screening of antioxidant and antimicrobial activity performed on 25 selected wild plant extracts revealed a satisfactory free radical scavenging and antimicrobial potential that could be of value in the management of oxidative stress. Further studies are recommended to explore novel and highly active natural antibacterial products.

Keywords: radical scavenging activity; antioxidant; oxidative stress; wild edible plant; natural product

1. Introduction

Many current commercial drugs and cosmetics are derived from natural sources. Naturally derived products are inherently better tolerated in the human body as opposed to synthetic chemicals, and, therefore, have a greater chance of being approved as drugs [1,2]. Modern therapeutics derived from natural products, particularly herbal-based drugs, averaged more than 70% of all drugs in use by the

year 1990 [3]. However, natural products have become a less significant source of leads and drugs over the past couple of decades. A dramatic decrease of about 50% in the number of new drug entities approved is observed. However, the global expenditures on drug research have doubled since 1991 [4]. This situation has changed with a shift back to natural-based products, which are believed to be better tolerated by the human body [5]. Nutritional factors are also recognized to play a key role in the prevention of, and therapy for, many ailments [6,7].

Palestine possesses a wide array of biodiversity, despite its limited area [8]. Although exploration for medicinal plants from Palestine for their antioxidant and antimicrobial benefits has improved over the last decade, a huge number of medicinal plants remain to be investigated [9,10]. Certain wild plants have acquired a reputation among Palestinians, since they are an integral component of the culture. A few years ago, Ali-Shtayah et al. [11] documented 100 wild edible plant species in an ethnobotanical survey of plant foods in the Northern West Bank. They identified 10 wild edible plants of notable cultural importance, which were further investigated in this study. The free radical scavenging activity of polar and non-polar fractions that were extracted from 25 wild edible plants (see Table 1) were investigated. Since free radicals of reactive oxygen species (ROS) are produced by the body to facilitate the killing of cancer cells [12] and microbes [13], assessing correlations between free radical scavenging activity and antimicrobial activity for the wild edible plants tested in this study was of great importance.

Free radicals are defined as chemical species possessing an unpaired active electron in the valence shell (hydroxyl HO, nitric oxide NO, etc.). Free radicals, which result from aerobic metabolic processes, are connected to a series of regulatory processes such as cell proliferation, apoptosis, and gene expression. They are ideally compensated for by an elaborate endogenous antioxidant defense system, so that normal cell functioning is characterized by an oxidant-antioxidant balance (redox homeostasis) [14]. However, the excessive generation of free radicals, or the acquisition of more from outside sources, can neutralize the defensive capability of the antioxidant systems to a large extent, which leads to damage in many vital biological components, such as DNA, cell proteins, enzymes, carbohydrates, and cellular membranes [15].

Oxidative stress is produced by the presence of unnecessary free radicals that are not neutralized by the antioxidant systems. It plays a vital pathological role in many chronic diseases and metabolic disorders, such as cancer [12,16], diabetes [17], cardiovascular disease [18], arthritis [19], ulcerative colitis [20], neural disorders [21], Alzheimer's disease [22], Parkinson's disease [23], cirrhosis [24], and aging [21]. Strengthening the antioxidant systems of the body by introducing antioxidants from outside sources can help eliminate excessive free radicals that cause oxidative stress, which impedes the progress of many oxidative stress-linked diseases. Free radicals are generated in the body by certain reactions that occur during normal cellular metabolism or by ionizing radiation (γ , X, and UV) [25], the biotransformation of dietary chemicals, and some diet-related components such as transient metal ions. Two approaches are recommended for restoring the free radical/antioxidant balance. The first is to remove factors that stimulate the production of free radicals. The second is to supply the body with antioxidants from outside sources. Recently, we are witnessing a growing interest in natural antioxidants that can be found plentifully in edible plants [26].

Thus, plants can serve as an excellent source of natural antioxidants and can offer therapeutic benefits for the treatment of oxidative stress through combinations of the wide range of active compounds found in them [27]. Natural products have been optimized to act on biological targets through a long natural selection process [28]. Thus, nature is rich in bioactive ingredients in general [28,29], and antioxidants are rich in particular [30]. Nature has been considered the best source of medicines for millennia [31], and most marketed drugs have natural origins [5].

Since the publication of the paper "The Seven Counties Study" by Keys et al. [32], interest in the Mediterranean diet has grown exponentially [33]. Despite its tiny size, Palestine, as part of the Mediterranean, enjoys a vast diversity of unique wild plants that have never been investigated. This uniqueness is due to its geographical location and its climate, which provides rich and generous

soil. This study tested the health benefits of wild edible plants by screening polar/non-polar fractions from 25 plants for their antioxidant and antibacterial activity. Correlations between the antioxidant and antibacterial activities of the extracts were assessed.

Table 1. List of tested plants and their extracts' activities.

Scientific Plant Name (Parts Used: Leaf, Pulp, Seed, Stem, etc.)	Local Name in Arabic	Polar Extract Free Radical Scavenging (EC ₅₀)/Antibacterial (% Inhibition by Extract of 5000 ppm Concentration)	Non-Polar Extract Free Radical Scavenging (EC ₅₀)/Antibacterial (IC ₅₀), ppm
<i>Majorana syriaca</i> (leaf + stem)	زعتري بري	1.6/22.5%	67.3/83.6
<i>Foeniculum vulgare</i> (leaf + stem)	شومر	52.1/34.1%	488.8/14.8
<i>Malva sylvestris</i> (leaf)	خبازة برية	10.1/29.3%	183.8/93.6
<i>Salvia palaestina</i> (leaf + stem)	ميرمية	12.5/41.6%	64.9/19.2
<i>Cyclamen persicum</i> (leaf)	عصا الراعي(زقويه)	35.2/24.8%	172.2/113.8
<i>Micromeria fruticosa</i> (leaf + stem)	عشبة الشاي	n.m. #1	56.1/5.3
<i>Arum palaestinum</i> (leaf)	لوف الفلسطيني	n.m. #1	3447.4/127.8
<i>Trigonella foenum-graecum</i> (seed)	حلبة	n.m. #1	1014.6/8.2
<i>Gundelia tournefortii</i> (stem)	عكوب	89.9/30.5%	1567.2/59.3
<i>Matricaria aurea</i> (leaf + stem)	البابونج الذهبي	84.3/11.3%	1864.5/99.9
<i>Centaurea dumulosa boiss</i> (leaf + stem)	مرار	55.8/43.9%	314.4/105.8
<i>Cichorium pumilum jacq</i> (leaf)	هندباء	50.9/23.2%	489.7/19.5
<i>Salvia hierosolymitana boiss</i> (leaf)	لسينه	12.1/48.8%	86.0/13.2
<i>Rumex patientia L.</i> (leaf)	سلق بري	>10,000/76.6%	233.0/28.9
<i>Ruta chalepensis</i> (leaf)	فيجن	21.5/94.7%	496.1/1.5
<i>Ceratonia siliqua</i> (fruit)	خروب	47.4/56.2%	180.2/39.0
<i>Urtica urens L.</i> (leaf + stem)	قريص حريق	590.9/n.d.	3736.0/62.2
<i>Portulaca oleracea L.</i> (leaf)	فرخينا	55.8/72.2%	1852.4/85.4
<i>Eryngium creticum lam</i> (leaf)	قرصنه	28.8/11.7%	99.6/30.5
<i>Chrysanthemum coronarium</i> (leaf + stem)	بسباس	n.m. #1	587.0/10.1
<i>Sinapis alba</i> (leaf)	(بري)عشبة الخردل	42.9/30.4%	739.1/102.5
<i>Asparagus aphyllus L.</i> (stem)	هليون	2803.2/23.0%	627.5/207.4
<i>Rumex acetosa</i> (leaf)	حميض	37.2/42.3%	496.1/85.4
<i>Teucrium chamaedrys</i> (leaf)	جعدة	9.2/2.8%	94.2/124
<i>Ephedra foeminea forssk</i> (stem)	علندا	10.8/52.9%	603.1/31.9

#1 not measured.

2. Materials and Methods

2.1. Chemicals and Instrumentation

Gallic acid standard and the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma Aldrich (Milwaukee, WI, USA).

2.2. Plants

The 25 species of wild edible plants were carefully selected. Ten of them possessed among the highest mean values for cultural importance include [11]: *Majorana syriaca*, *Foeniculum vulgare*, *Malvasylvestris*, *Salvia palaestina*, *Cyclamen persicum*, *Micromeria fruticosa*, *Arum palaestinum*, *Trigonella foenum-graecum*, *Gundelia tournefortii*, and *Matricaria aurea*. The other 15 plant species were *Centaurea dumulosa boiss*, *Cichorium pumilum jacq*, *Salvia hierosolymitana boiss*, *Rumex patientia* L., *Ruta chalepensis*, *Cerantonia siliqua*, *Urtica urens* L., *Portulaca oleracea* L., *Eryngium creticum lam*, *Chrysanthemum coronarium* L., *Sinapis alba*, *Asparagus aphyllus* L., *Rumex acetosa*, *Teucrium chamaedrys*, and *Ephedra foeminea forssk*.

2.3. Plant Extraction

The plants were collected from the mountains of the Nablus region and Kabul mountain (north Galilee) during the period May 2018 to April 2019. They were identified and authenticated by Prof. Khaled Sawalha of the Biology Department at Al-Quds University. Three batches from each species were collected and mixed together to prepare the representative material of each plant. The specimens were washed with distilled water and dried in the shade for one month. Then, they were ground up and packed in the tubes of Soxhlet (Corning, Inc., NY, USA). In a round-bottomed flask, a mixture of solvents composed of 60 mL of water, 30 mL of ethanol, 30 mL of ethyl acetate, and 30 mL of hexane was introduced. The aim of using a mixture of solvents is maximizing natural products extraction into polar and non-polar layers. After the extraction was completed (following 3 cycles), the polar and non-polar phases were separated with a separation funnel. Both the polar and non-polar extracts were filtered by passing the solutions through 0.2- μ m disposable filters. The solvents from each extract were evaporated in an oven under reduced pressure. The polar extracts (free of solvents) were dissolved in water, while the non-polar extracts were dissolved in dimethyl sulfoxide (DMSO) both at a concentration of 20,000 ppm. The prepared solutions were subjected to free radical scavenging tests using the DPPH assay. Microdilution tests were also conducted to determine the IC₅₀s of the extracts against *S. mutans*.

2.4. Free Radical Scavenging Activity

There are several assays that are used to determine antioxidant activity of plant extracts. The most used one is based on free radical scavenging of the antioxidant to the stable chromogenic radical scavenge DPPH (2,2-diphenyl-1-picryl hydrazyl). The reduction of DPPH by the hydrogen donor antioxidants cause decolorization of the dark purple color of DPPH and, as a result, the absorption decreases. The method is simple, rapid, accurate, and inexpensive. Currently, more than 17,000 articles were cited using this simple and straightforward method [34]. The method was first invented by Blois [35] and then modified by many investigators [36]. In the current study, the free radical scavenging activity of the various extracts was measured by microdiluting the DPPH assay, with some modifications. The microdilution of the DPPH assay was performed using two-fold serial dilution in ethanol. The tests were carried out in 96-well, flat-bottomed microtitration plates. The absorbance of the solution was measured at 517 nm and converted into a percentage of free radical scavenging activity using Equation (1).

$$\text{Scavenging activity \%} = 100 \times \{1 - [(A_{\text{sample}} - A_{\text{blank}_1}) / (A_{\text{control}} - A_{\text{blank}_2})]\} \quad (1)$$

where the A_{sample} is the absorbance of the mixture (plant extract and DPPH), A_{blank_1} is the absorbance of the plant extract, A_{control} is the absorbance of the ethanolic solution of DPPH, and A_{blank_2} is the absorbance of the blank (solvent).

Gallic acid was used as a positive control. The free-radical scavenging was expressed in terms of EC_{50} (the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%). The EC_{50} value for each plant extract was determined by extracting the value from the equation for the linear part of the graph. We substitute 50% for the y value while calculating the concentration value of the x -axis.

2.5. Antibacterial Activity

The microdilution test was used to determine the 50% inhibitory concentration (IC_{50}) of the different extracted plant samples. A broth microdilution assay was performed using two-fold serial dilution in brain heart infusion (BHI) broth. The test was carried out in 96-well, flat-bottomed microtitration plates. The cell suspension was prepared in BHI broth with an optical density equivalent to the 0.5 McFarland standard and diluted 1:100 in BHI broth to obtain a final concentration of 5×10^5 clone-forming units per milliliter (CFU/mL). Controls of broth only and broth with bacteria without any of the antibacterial agents were also included in each plate. One hundred μL of antibacterial agent was put in the first microplate well and serially diluted in BHI broth. One hundred μL , corresponding to 5×10^5 CFU/mL, was added to all the wells. The plates were incubated at 37°C for 24 h. For more details regarding the cultivation condition, see our recent publications [1,37]. Tetracycline was used as a positive control for *S. mutans*. The IC_{50} was defined as the concentration able to inhibit the growth of bacteria in the quadruplicate wells. The absorbance of the solution was measured at 620 nm and converted into a percentage of bacterial growth inhibition, using the Equation (2).

$$\text{Antibacterial activity\%} = 100 \times \{1 - [(A_{\text{sample}_{24}} - A_{\text{sample}_0}) / (A_{\text{control}_{24}} - A_{\text{control}_0})]\} \quad (2)$$

where $A_{\text{sample}_{24}}$ is the absorbance of the mixture (plant extract and bacteria) after 24 h, A_{sample_0} is the absorbance of the mixture (plant extract and bacteria) at time 0, $A_{\text{control}_{24}}$ is the absorbance of the untreated bacteria wells after 24 h, and A_{control_0} is the absorbance of the untreated bacteria wells at time 0.

The antibacterial IC_{50} for each plant extract was determined by extracting the value from the equation of the linear part of the graph. The negative control was DMSO solvent and, as reported in previous publications [1,37], its minimum inhibitory concentration (MIC) value is 25% (v/v).

2.6. Model Assessments

Parameters such as Matthew's correlation coefficient (MCC) [38], accuracy, precision, enrichment factors, and the area under the ROC curve (AUC) were used to assess the quality of the antibacterial/free radical scavenging correlation models.

Equation (3): Matthew's correlation coefficient (MCC)

$$\text{MCC} = \frac{(PN) - (P_f N_f)}{\sqrt{(N + N_f)(N + P_f)(P + N_f)(P + P_f)}} \quad (3)$$

Equation (4): Accuracy

$$\text{Accuracy} = (P + N) / (P + N + P_f + N_f) \quad (4)$$

Equation (5): Precision

$$\text{Precision} = P / (P + P_f) \quad (5)$$

Equation (6): Enrichment factor

$$\text{Enrichment factor} = T_{FRS}/T_{RS} \quad (6)$$

where P , N , P_f , and N_f are the numbers of true positive, true negative, false positive, and false negative predictions, respectively. T_{FRS} is the percentage of actives when using the free radical scavenging threshold criterion and T_{RS} is the percentage of actives by random selection.

2.7. Statistical Analysis

All data are presented as the average of three to four experiments, and error bars display the standard errors of deviations. All statistical analyses were conducted using Microsoft EXCEL 2013 with its Data Analysis add-in (v16.0, Microsoft, Redmond, WA, USA). The dose-response curves for scavenging activity were plotted based on the percentage of scavenging and the concentration. The EC_{50} values for free radical scavenging were determined with linear regression analysis. Reliability lowers with a decrease in the R^2 value. Differences between the groups were evaluated by applying a one-way analysis of variance (ANOVA). A p -value of less than 0.05 is considered statistically significant.

3. Results and Discussion

Twenty-five wild edible plants were studied for their free radical scavenging and antibacterial activity against *S. mutans*. Antibacterial potential was first verified by screening all the extracts (polar and non-polar fractions) for their activity, using one concentration of 250 ppm. The polar fractions were less active than the non-polar fractions. Column 3 of Table 1 depicts the EC_{50} of the free radical scavenging and the percentage of inhibition against *S. mutans* at a 5000-ppm concentration of the polar fraction. The extracts that exhibited a percentage of bacterial inhibition above 60% were tested in the second round at lower concentrations in a dose-response manner to extract their IC_{50} values. The antibacterial IC_{50} values of the polar fraction of the three plants *Ruta chalepensis*, *Rumex patientia* L., and *Portulaca oleracea* L. are 1750 ppm, 3925 ppm, and 4133 ppm, respectively. Column 4 of Table 1 depicts the EC_{50} of the free radical scavenging and the IC_{50} against *S. mutans*. Eight extracts (non-polar fractions) were found to possess relatively high antibacterial IC_{50} values of less than 20 ppm such as *Foeniculum vulgare*, *Salvia palaestina*, *Micromeria fruticose*, *Trigonella foenum-graecum*, *Cichorium pumilum jacq*, *Salvia hierosolymitana boiss*, *Ruta chalepensis*, and *Chrysanthemum coronarium*.

Rules-based analysis using Matthew's correlation coefficient (MCC) scores and enrichment factors as criteria for evaluating the models' efficiency revealed that the plant extracts whose EC_{50} for free radical scavenging <100 ppm showed some degree of enrichment toward being more active as an antibacterial agent against *S. mutans* (see Table 2). The values for the enrichment factor, the MCC, accuracy, and precision were 4.1, 0.217, 0.68, and 0.5, respectively. These findings are of high importance for screening projects. A four-fold order of enrichment toward more activity against *S. mutans* for the extracts with an EC_{50} of free radical scavenging \leq 100 ppm could save plenty of time and could save many in screening projects. Considering all the results can lead to the conclusion that there is no correlation between antibacterial activity against *S. mutans* and free radical scavenging (as shown in Figure 1, R^2 equals 0.0538), and also for the eight most active non-polar plant extracts (as shown in Figure 2, R^2 equals 0.0469). Three of the eight active plant extracts (*Salvia palaestina*, *Micromeria fruticose*, and *Salvia hierosolymitana boiss*) show relatively high free radical scavenging, and four others (*Foeniculum vulgare*, *Cichorium pumilum jacq*, *Ruta chalepensis*, and *Chrysanthemum coronarium*) show medium free radical scavenging, while the remaining extract (*Trigonella foenum-graecum*) shows relatively low free radical scavenging. The results show that differences in antibacterial activity among the non-polar extracts cannot always be attributed to antioxidant levels but could be associated with inhibitory effects via other mechanisms of action [39] (inhibition or regulation of enzymes involved in cell wall biosynthesis, nucleic acid metabolism and repair, protein synthesis, or disruption of the membrane structure).

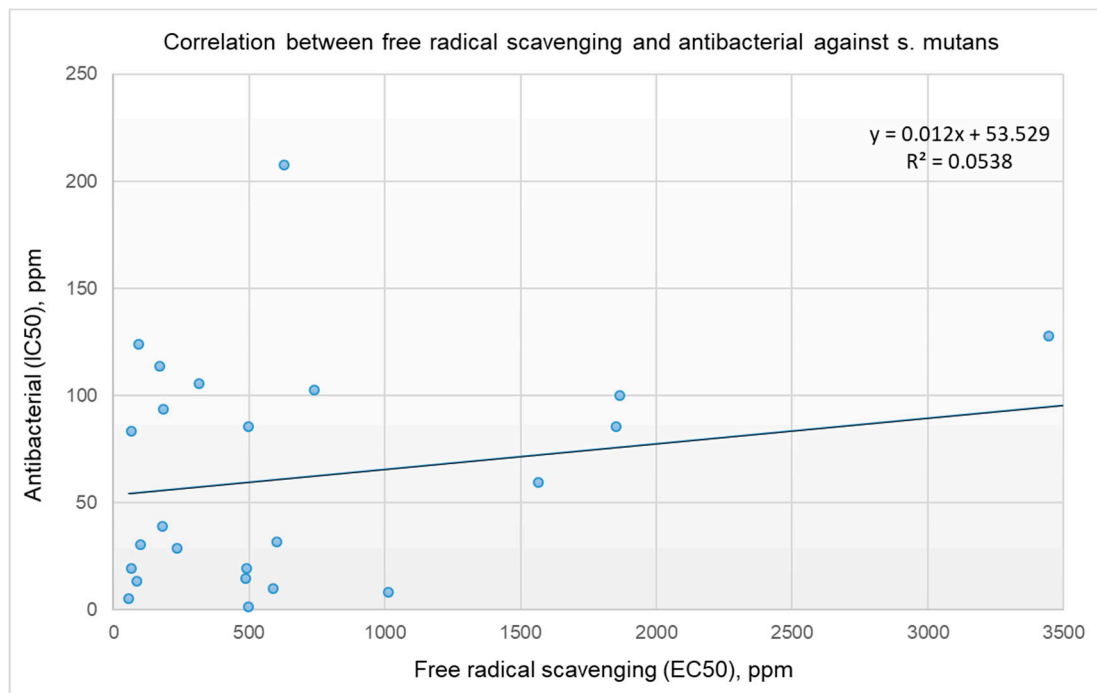


Figure 1. Correlations between the EC₅₀ for free radical scavenging and antibacterial activity (IC₅₀) for the non-polar extracts.

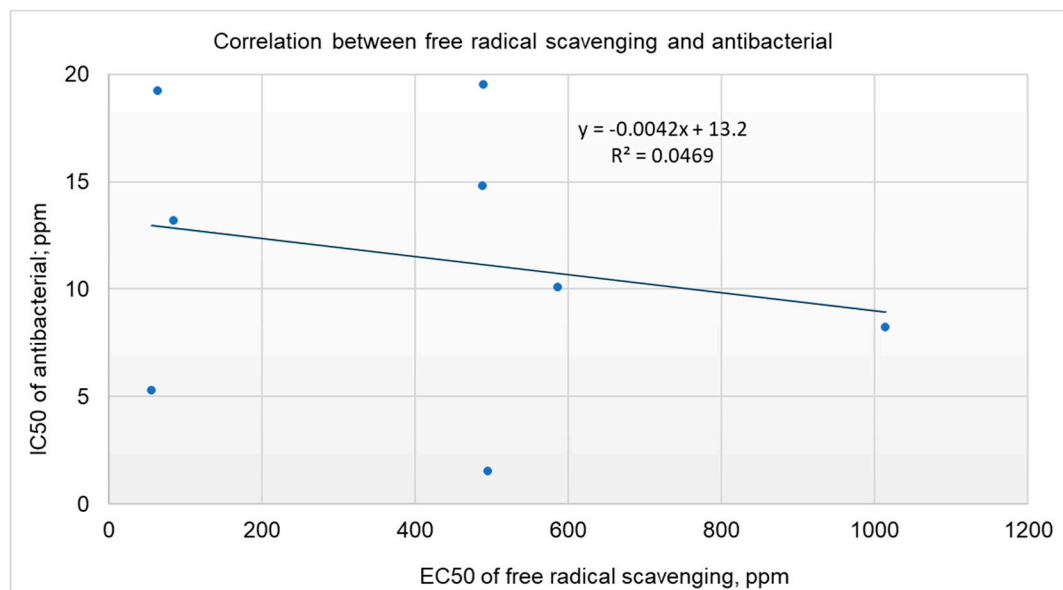


Figure 2. Correlations between antibacterial activity (IC₅₀) and the EC₅₀ for free radical scavenging for the eight most active plant extracts against *S. mutans*.

Figure 3 depicts the enrichment plot and Figure 4 shows the receiver operating characteristic (ROC) plot for the antibacterial/ free radical scavenging correlation model. The area under the curve (AUC) that was attained for the current model, as shown in Figure 4, is 0.661, which means that the model is very poor. This indicates a very weak correlation between antibacterial and free radical scavenging. The enrichment plot, which is shown in Figure 3, illustrates how quickly antibacterial extracts of plants can be identified when they are sorted based on their free radical scavenging activity. In-depth analysis revealed that the shape of the figure fits well with the conclusions drawn from the

detailed analysis of Table 2, which shows that non-polar plant extracts with an EC_{50} of free radical scavenging <100 ppm have a four-fold order of enrichment toward more activity against *S. mutans*.

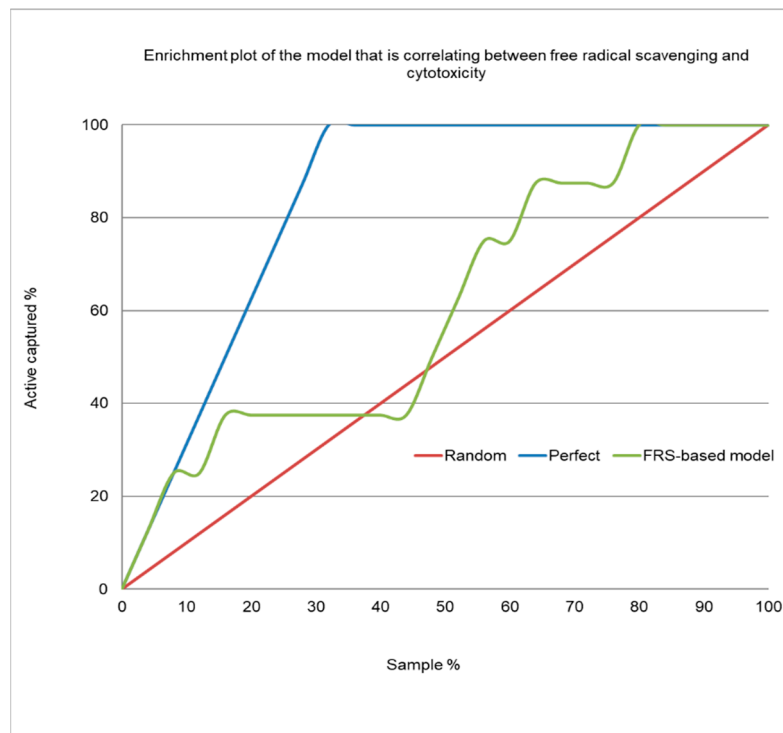


Figure 3. Enrichment plot of the prediction model for the antibacterial activity of the plant extracts, based on their free radical scavenging activity (non-polar extracts).

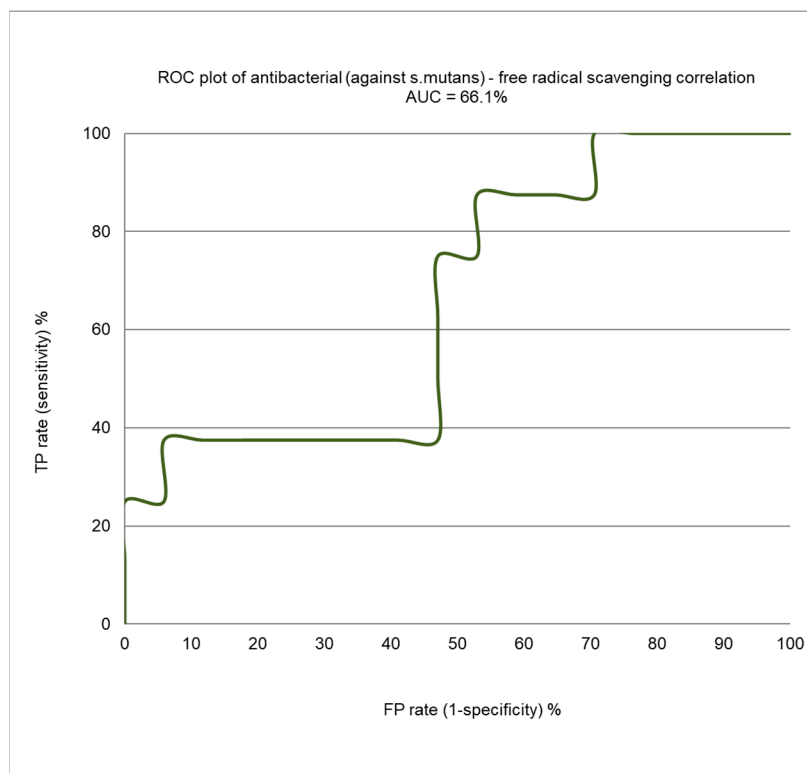


Figure 4. A receiver operating characteristic (ROC) curve showing the performance of the antibacterial/free radical scavenging correlation model.

Table 2. Matthew’s correlation coefficient (MCC) scores, precision, accuracy, and enrichment factors as criteria for evaluating the prediction models.

EC ₅₀ Cutoff of Free Radical Scavenging (<)	100 ppm	500 ppm	1000 ppm	No Limit
No. active plants (true positive) ^{#1}	4	6	7	8
No. inactive plants (false positive) ^{#2}	3	9	11	17
No. inactive plants (true negative) ^{#3}	14	8	6	-
No. active plants (false negative) ^{#4}	4	2	1	-
Precision	0.5	0.4	0.37	0.32
Accuracy	0.68	0.56	0.48	0.32
Enrichment factor	4.1	1.66	1.31	1.0
MCC	0.217	0.210	0.184	0.0

^{#1} Number of plant extracts that have an EC₅₀ for free-radical scavenging lower than the indicated threshold and an IC₅₀ for antibacterial activity lower than 20 ppm. ^{#2} Number of plant extracts that have an EC₅₀ for free-radical scavenging lower than the indicated threshold and an IC₅₀ for antibacterial activity greater than 20 ppm. ^{#3} Number of plant extracts that have an EC₅₀ for free-radical scavenging greater than the indicated threshold and an IC₅₀ for antibacterial activity greater than 20 ppm. ^{#4} Number of plant extracts that have an EC₅₀ of free-radical scavenging greater than the indicated threshold and an IC₅₀ for antibacterial activity lower than 20 ppm.

When the eight active plants were searched through electronic databases, including PubMed, we found a huge number of relevant reports documenting their antibacterial potential on different statins, except for *Salvia hierosolymitana*. For example, the antimicrobial activity of *Foeniculum vulgare* has been extensively reviewed [40]. The antibacterial activity of its essential oil against *S. mutans* showed an MIC and an MBC of 8.4 and 14.9 ppm, respectively. *Salvia palaestina* was also recently investigated by us for its antioxidant and antimicrobial potential [41]. The antimicrobial activity was examined by the disc diffusion method, and 5 µL (100%) of the essential oil was found to exceed the activity of gentamicin in the case of *S. aureus*, while it was nearly as effective as gentamicin against *E. coli*. Furthermore, according to both SEM and crystal-violet staining data, the essential oil of *Micromeria fruticosa* was found to exert an inhibitory effect on *S. mutans* biofilm formation [42]. *Trigonella foenum-graecum* (fenugreek) was also found to be effective in amounts of 20 mm and 19 mm against *E. coli* and *Staphylococcus*, respectively [43]. *Cichorium pumilum*, which is widely used as a traditional medicinal herb, showed antibacterial activity in vitro with agar disc diffusion and agar well diffusion methods against 10 different bacterial species [44]. The antimicrobial activity of *Ruta chalepensis* L. was tested against four pathogenic bacteria—*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*—and the findings indicate possible applications for the treatment of various infectious and noninfectious diseases [45]. The antibacterial effects of essential oil of *Chrysanthemum coronarium* demonstrated pronounced activity against gram-positive strains of bacteria: *Bacillus subtilis* (19 mm), *Staphylococcus aureus* (20 mm), *Staphylococcus epidermidis* (18 mm), *Escherichia coli* (9 mm), and *Pseudomonas aeruginosa* (12 mm), which were assessed by measuring the zone of inhibition [46]. Another unique aspect of this study is the scanning of all the 25 plant extracts at the same lab by using the same procedures for testing their antioxidant and antibacterial activities.

4. Conclusions

For this study, free radical scavenging was assessed by a DPPH assay, while antibacterial activity was measured by a microdilution assay. Eight wild plant extracts (non-polar fractions) were found to possess antibacterial IC₅₀ values of less than 20 ppm, including *Foeniculum vulgare*, *Salvia palaestina*, *Micromeria fruticosa*, *Trigonella foenum-graecum*, *Cichorium pumilum jacq*, *Salvia hierosolymitana boiss*, *Ruta chalepensis*, and *Chrysanthemum coronarium*. In addition, we raise a question regarding the correlation between free radical scavenging and antibacterial activity. Answering this question might be helpful for making the screening process more efficient. Looking at all the results together can deceive and lead to the conclusion that there is no correlation between antibacterial activity against *S. mutans* and free radical scavenging (R^2 equals 0.0538). However, an in-depth analysis of the results revealed that the extracts of plants that exhibited an EC₅₀ of free radical scavenging of ≤100 ppm

showed some degree of enrichment (four orders of magnitude) toward more antibacterial activity. These findings are of high importance for screening projects. A four-fold order of enrichment could save plenty of time and many in screening projects. The antibacterial activity of the extracts could be attributed to various mechanisms of action. The highly active free radical scavenging extracts might follow one mechanism of action, while the extracts that are marked by low-medium free radical scavenging might act through another mechanism of action. This hypothesis could explain the enrichment that exist in extracts having EC₅₀ of free radical scavenging of ≤100 ppm.

Our next study will aim to discover novel and highly active natural antibacterial products, using a novel in-house bioassay guided fractionation approach [1,28,31,37] and in-silico based modeling techniques [29,47–50] to identify natural products that exhibit antibacterial activity against *S. mutans*.

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