horticulturae

MDPI

Article

GC-MS Characterization, In Vitro Antioxidant, Antimicrobial, and In Silico NADPH Oxidase Inhibition Studies of *Anvillea radiata* Essential Oils

Ghada Beniaich [1], Ouattar Hafsa [1], Imane Maliki [2], Yousef A. Bin Jardan [3], Abdelfattah El Moussaoui [4], Mohamed Chebaibi [5], Abdelkrim Agour [6], Otmane Zouirech [6], Hiba-Allah Nafidi [7], Farid Khallouki [8], Mohammed Bourhia [9,*] and Mustapha Taleb [1]

1 Laboratory of Molecular and Environmental Organometallic Materials Engineering (LIMOM), Faculty of Sciences Dhar El Mahraz, University of Sidi Mohamed Ben Abdellah, Fez 30050, Morocco
2 Laboratory of Health and Environment, Department of Biology, Moulay Ismail University, Meknes 50050, Morocco
3 Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
4 Laboratory of Biotechnology, Environment, Agrifood and Health, Faculty of Sciences Dhar El Mahraz, University of Sidi Mohamed Ben Abdellah, Fez 30050, Morocco
5 Laboratory Biomedical and Translational Research, Faculty of Medicine and Pharmacy of the Fez, University of Sidi Mohamed Ben Abdellah, Km 22, Road of Sidi Harazem, Fez 30050, Morocco
6 Laboratory of Natural Substances, Pharmacology Environment, Modeling, Health and Quality of Life, Faculty of Sciences Dhar El Mahraz, University Sidi Mohamed Ben Abdellah, Fez 30000, Morocco
7 Department of Food Science, Faculty of Agricultural and Food Sciences, Laval University, 2325 Rue de l'Université, Quebec City, QC G1V 0A6, Canada
8 Biology Department, FSTE, University Moulay Ismail, Errachidia 52000, Morocco
9 Higher Institute of Nursing Professions and Technical Health, Laayoune 70000, Morocco
* Correspondence: bourhiamohammed@gmail.com

Abstract: *Anvillea radiata* is a medicinal plant that has been used in traditional phytotherapy in North Africa as a treatment for various illnesses. In this study, we aim to explore the antioxidant, antifungal, and antibacterial effects of essential oils of *Anvillea radiata* (EOAR) collected in Morocco. EOAR was extracted by the hydrodistillation method, and the phytochemical identification was carried out by gas chromatography-Mass Spectrometry (GC/MS). The antioxidant capacity was evaluated by the 2.2-diphenyl-1-picrylhydrazyl (DPPH) method, ferricyanide method (FRAP) as well as total antioxidant capacity (TAC). Antifungal and antibacterial properties were determined by use of the disc diffusion and minimum inhibitory concentration assays. The microbial strains used in the antimicrobial evaluation were: *Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 9606), *Fusarium oxysporum* (MTCC 9913), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATB 97/BGM), *Staphylococcus aureus* (ATCC 6633), *Bacillus subtills* (DSM 6333), and *Escherichia coli* (ATB 57/B6N). Based on in silico simulations, the inhibitory power of EOAR against nicotinamide adenine dinucleotide phosphate oxidase (NADPH) was evaluated. The yield of the oil was 0.96% wherein 12 compounds were identified including α-cuprenene (33.48%) camphor (21.41%) and α-himachalene (15.88%) as major compounds. The antioxidant capacity showed an IC50 of 32.36 µg/mL (DPPH) and an EC-50 value of 64.60 ± 3.71 µg/mL in the FRAP assay. The total antioxidant capacity showed a concentration of 977.51 ± 22.38 µg AAE/mg (TAC). As for the antimicrobial effects, the inhibition diameter of the studied bacteria ranged from 23.50 ± 2.31 to mm 29.50 ± 2.21 mm, while for fungi, ranged from 25.12 ± 2.82 mm to 11.42 ± 1.90 mm. Minimum Inhibitory Concentration (MIC) ranged from 12.71 ± 1.59 µg/mL to 23.53 ± 0.78 µg/mL for bacterial strains and 10.31 ± 1.34 µg/mL to 22.75 ± 1.06 µg/mL for fungal strains. In silico, among all *Anvillea radiata* essential oils analyzed, the sesquiterpene γ-dehydro-ar-himachalene, monoterpenoid phenol carvacrol, as well as sesquiterpene α-cadinene were the most active compounds against NADPH oxidase with a glide score of −6.233, −6.082, and −5.057 Kcal/mol, respectively. Taken together, these data showed that EOAR exhibited enormous significance as an antioxidant, antifungal, and antibacterial agent.

Citation: Beniaich, G.; Hafsa, O.; Maliki, I.; Bin Jardan, Y.A.; El Moussaoui, A.; Chebaibi, M.; Agour, A.; Zouirech, O.; Nafidi, H.-A.; Khallouki, F.; et al. GC-MS Characterization, In Vitro Antioxidant, Antimicrobial, and In Silico NADPH Oxidase Inhibition Studies of *Anvillea radiata* Essential Oils. *Horticulturae* **2022**, *8*, 886. https://doi.org/10.3390/horticulturae8100886

Academic Editor: Alessandra Carrubba

Received: 31 August 2022
Accepted: 24 September 2022
Published: 28 September 2022

Keywords: medicinal plant; natural products; antifungal; antibacterial; free radicals; carvacrol; cuprenene

1. Introduction

Medicinal plants produce a wide variety of secondary metabolites which have become the reference in terms of pharmacopoeia and are still the subject of a myriad of in vivo and in vitro studies [1,2]. Plants are an essential source for discovering novel medicines and remain the mainstay of many developing countries. At least 80% of Africa's rural population use medicinal plants for their health care [2,3]. In North Africa, there are about 5000 species and subspecies that possess medicinal properties [4]. *Anvillea radiata* Coss (Nogd) belongs to the family Asteraceae [5], which is used in traditional phytotherapy as a treatment for certain diseases [6–8]. *Anvillea radiata* is also a rich source of polyphenols with potent antioxidant, antihypertensive and antidiabetic activities [9].

Since the beginning of the 20th century, free radicals and antioxidants have been the most widely used terms in scientific research, characterized by their ability to oxidize into other molecules. Their impact on health was only studied by biologists in the early 1960s through work on vitamins and flavonoids, followed by work on ascorbic acid in late 1970 [10–12]. The accumulation of reactive oxygen species results in the appearance of often irreversible cellular and tissue damage, the most vulnerable biological targets of which are proteins, lipids, and deoxyribonucleic acid [13–16].

An important therapeutic advance for human health, antibiotics have long been used to reduce mortality and morbidity rates around the world. However, due to the misuse of these antimicrobial drugs and their growing use, new microbial populations with resistance to some types of antibiotics have emerged [17]. Certain strains are capable of simultaneously re-establishing multidrug resistance to several antibiotics, giving rise to bacteria known as multidrug-resistant (MDR) bacteria [18]. Numerous investigations have been conducted to develop substitute molecules that are effective against various infectious diseases in response to this issue. Medicinal and aromatic plants can be used as a source of bioactive compounds that could mitigate infections and may replace synthesized forms [17,19–22].

Essential oils (EOs) are a group of aromatic and isoprene secondary metabolites, which are obtained by use of steam distillation and are often identified in several families of aromatic plants [23,24]. EOs are known for their pharmacological role as an antioxidant, antibacterial, and insecticide [25–29].

This study aimed to characterize the chemical composition of Moroccan *Anvillea radiata* essential oil by use of GC-MS, and evaluate its antioxidant, antibacterial, and antifungal activities. In silico model for NADPH oxidase inhibition is also approached.

2. Material and Method

2.1. Chemicals Used

Ammonium molybdate, butylated hydroxytoluene (BHT), sodium phosphate, quercetin, triphenyl-tetrazolium chloride (TTC), potassium-ferricyanide ($K_3Fe(CN)_6$), 2.2-diphenyl-1-picrilhidrazil (DPPH), methanol and trichloracetic acid (TCA) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Material

The plant was harvested during the period of maximum flowering (March, 2022), in a desert area with geographic coordinates (4°16′17″ N 31°12′17″ W; 748 m). The plant was identified by a botanist and given a voucher specimens RA/03/MarAr22.before being deposited at the Herbarium of the Department of Biology, University of Sidi Mohamed Ben Abdellah. The plant aerial part was dried by use of an oven at 40 °C for a few days before being ground into a fine powder using an electric grinder. By use of a Clevenger apparatus, EOAR extraction was done by mixing 200 g of the powder with one liter of

distilled water. Afterward, EOARs were recovered and stored in stained vials at 4 °C until further use [30,31]. The extraction yield was calculated according to the following equation:

$$Y\ (\%) = (WH/WP) \times 100$$

where Y(%): Extraction yield in percent (%); WH: Weight of extracted essential oils; WP: Weight of powder used in the extraction.

2.3. GC-MS Identification of A. radiata Essential Oil

A Gas chromatography UltraStable vector builder (GC-VB-UTRA) chromatographic system characterized by a VB-5 colon with an internal diameter (250 µm), length (30 m) and film thickness (250 µm) was used to characterize the phytochemical compounds of EOAR. The following operational parameters were set as follows: helium as the carrier gas, with a flow rate of 1400 µL/min; *Temperature Program*, from 50 °C ramp 4 °C/min to 190 °C before spending 20 min at 300 °C. The injection volume was set to 1 µL. The other settings included also an Electronic Impact type ionization (70 eV) at 200 °C. The temperature interface was set at 300 °C. By calculating their retention indices relative to a homologous series of n-alkanes and matching their registered mass spectra with those documented in referenced databases, the phytochemical components of EOAR were identified using the NIST-MS library.

2.4. Evaluation of Antioxidant Potential of EOAR

2.4.1. DPPH Test

This test was done by preparing free radicals (DPPH) at a concentration of about 4000 µg in 100 mL of methanol, while EOAR was prepared in methanol at different concentrations from 0.001 mg to 0.1 mg/mL. Next, 100 µL of EOAR was added to 750 µL of DPPH solution. Subsequently, the mixture was incubated at 30 °C for 30 min. Methanol was used as a negative control, while BHT and Quercetin were used as a positive control. The optical density was measured at λ = 517 nm, and the percentage inhibition was calculated according to the mathematical equation [32,33]:

$$IP\ (\%) = \frac{CT - SD}{CT} \times 100$$

where *IP*: percentage inhibition; *SD*: sample optical density and *CT*: negative control optical density.

2.4.2. FRAP Test

FRAP test was performed as described by Elmoussaoui et al. [34]. Briefly, 500 µL of phosphate buffer, 100 µL of EOAR prepared in methanol (10 µg/mL to 100 µg/mL) and 500 µL of potassium-ferricyanide were mixed in methanol and incubated for 25 min. Next 500 µL of TCA (10 mg/mL), 500 µL of distilled water and 100 µL of $FeCl_3$ (0.1 mg/mL) were mixed with the incubated solution. The optical density (OD) was read against a control without the sample. An effective concentration of 50% was used to express the results (EC50).

2.4.3. TAC Test

TAC test was carried out by mixing 30 µL of various concentrations of EOAR (100, 250, 500, 1000 µg/mL) with 1000 µL of a solution comprising sulphuric acid (0.2 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). Next, the mixture was then incubated at 96 °C for 90 min. The optical density was then measured at 695 nm against a control [31,35]. TAC was given in µg of ascorbic acid equivalent (µg AAE/mg) per mg of EO [31].

2.5. Antimicrobial Activity

2.5.1. Stains Used for Testing

In this study, *Aspergillus niger* MTCC 282, *Fusarium oxysporum* MTCC 9913, *Escherichia coli* ATB-57-B6N, *Staphylococcus aureus* ATCC 6633, *Escherichia coli* ATB-97-BGM, and *Bacillus subtilis* DSM 6333, *Aspergillus flavus* MTCC 9606, *Candida albicans* ATCC 10231 were used to conduct antimicrobial potential of EOAR. These strains were provided by the laboratory of microbial biotechnology at the Faculty of Medicine and Pharmacy, Fez.

2.5.2. Antimicrobial Power on Solid Medium

The disc diffusion method was utilized to assess the antifungal and antibacterial activity of EOAR on solid medium [30,36]. In Petri plates containing nutrient broth (NB) medium, bacteria were cultivated, whereas malt extract agar (MEA) medium was used for the growth of fungi. A few colonies were aseptically seeded in 0.9 mg/mL of sodium chloride from the fresh bacterial culture at a density of 0.5 McFarland (10^7–10^8 CFU/mL), while the yeast solution was estimated at 10^6 CFU/mL. Discs with six millimeters in diameter were placed on the agar surface of Petri dishes after being soaked in 10 µL of EOAR, while discs soaked in kanamycin served as positive controls [14,31]. After incubation of Petri plates at 37 °C for bacteria and 30 °C for fungi, results were noted [37,38].

2.5.3. Antimicrobial Power on Liquid Medium

In the present work, the minimum inhibitory concentration (MIC) of EOAR against bacterial and fungal strains was calculated using the microdilution method. Briefly, EOAR solution was diluted in 5% DMSO, meanwhile, the positive controls, which included kanamycin, and fluconazole, were diluted in either extract–peptone–glycerol or Mueller Hinton agar media. After that, 100 µL of each medium was placed into microplate wells. After that, one hundred microliters of material from each fraction were deposited into the first well. Next, a micro-dilution was performed by diluting the sample by a factor of 2 in each well, except for the last well, which served as the positive control for growth. Inoculation was carried out by pouring fifty microliters of the suspension into each well of the microplate. The first well, which served as a control for the absence of growth, did not receive inoculum.

Using the colorimetric method (TTC 0.20 percent (w/v)), the MIC was determined by direct observation of the growth in the wells following incubation times of 24 h for bacteria at 37 °C, 48 h for yeast, and seven days for fungi at 30 °C [30,31,38,39].

2.6. Molecular Docking

All essential oil identified in *Anvillea radiata* is downloaded from the PubChem database in SDF format. Then, they are prepared by LigPrep tool in the Maestro 11.5 version of the Schrödinger Software program using the OPLS3 force field. A maximum of 32 stereoisomers were produced for each ligand after the ionization states at pH 7.0 ± 2.0. The three-dimensional crystal structure of NADPH oxidase is downloaded in PDB format from the protein data bank using the following PDB ID: 2CDU. The structure is prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v11.5. The minimization of the structure was carried out using OPLS3 force field. The receptor grid is setting at the following coordinates: X = 19.853, Y = −6.431 and Z = −0.896. When the volumetric spacing performed is 20 × 20 × 20, SP flexible ligand docking was carried out in Glide of Schrödinger-Maestro v 11.5 [40].

2.7. Statistical Analysis

All the tests were done in triplicate and results were reported as mean ± standard deviation. Shapiro-Wilks method was used to verify the normality test, and the Levene test was used to assess the assumption of homogeneity of variance. Tukey's statistical test served as the posthoc analysis for multiple comparisons. When $p < 0.05$, a statistically significant difference was considered.

3. Results and Discussion

3.1. EOAR GC/MS Analysis

The extraction yield of EOAR was 1.08 ± 0.27%, which is higher than the yields found by El Hanbali and co-authors for the essential oil of *A. radiata* as they found a recovery of about 0.5% [41]. The GC/MS identification revealed 12 compounds in EOAR wherein α-Cuprenene (33.48%), camphene (21.41%) and α-Himachalene (15.88%) were dominant (Figure 1). EOAR is rich in sesquiterpene hydrocarbon (65.83%) followed by monoterpene oxygen-containing derivatives (23.95%). Compared to our results, El Hanbali and co-authors [41] identified more than 20 compounds in EOAR constituting 88% of its total mass. 6-oxocyclonerolidol (66.6%), 6-hydroxycyclonerolidol (11.4%) and transchrysanthenyl acetate (3.9%) (Table 1) were reported as major phytoconstituents. The qualitative and quantitative variation between these essential oils may be due to the variation in climatic conditions, harvest period as well as the method used in the extraction and identification [42,43].

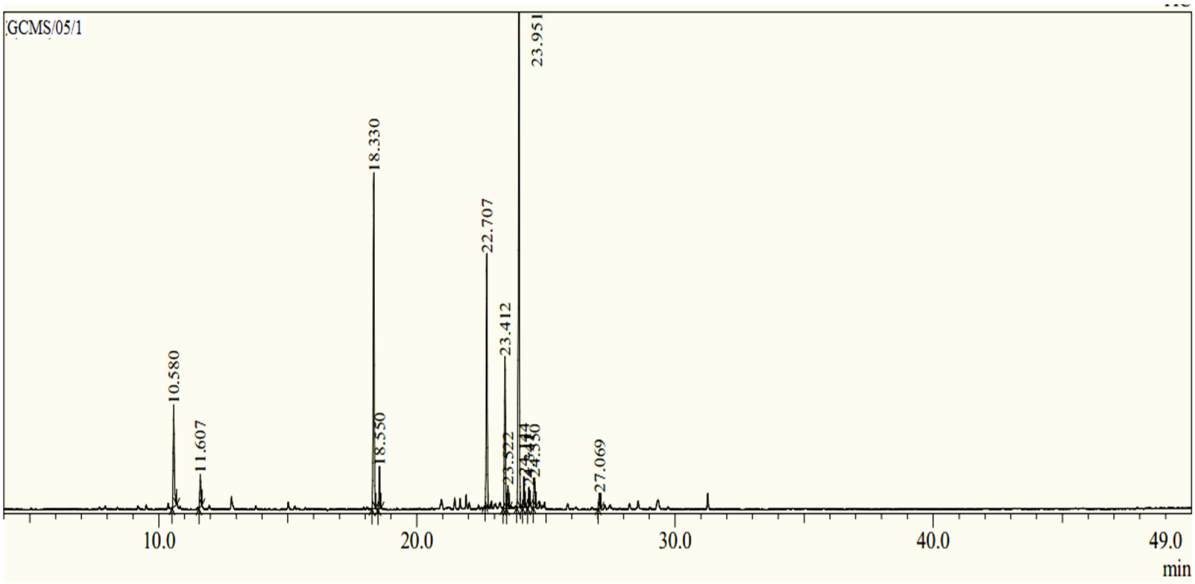

Figure 1. Gas chromatographic analysis of EOAR by GC/MS.

3.2. Antioxydant Activity of EOAR

The DPPH scavenging activity results by EOAR revealed a dose-dependent response (Figure 2A). Notably, EOAR showed inhibition of DPPH free radicals with an IC50 was about 32.36 µg/mL, whereas IC50 values for positive controls BHT and Quercetin were determined to be 17.83 µg/mL and 21.98 µg/mL (Figure 2B) respectively. A lower IC50 value indicated higher antioxidant power of the sample [33,44]. Kandouli et al. found an IC50 of about 45 µg/mL for *Anvillea radiata* [8]. Furthermore, antioxidant power EOAR is higher than that of *Dittrichia viscosa* essential oils with an IC50 value of 1.36 mg/mL [45], and lower than that of *Juniperus thurifera* essential oils, with an IC50 value of 24 ± 0.71 µg/mL [46].

As shown in Figure 2C, the evaluation of the antioxidant potency of EOAR by the FRAP method also revealed a dose-response relationship, as the concentration of EOAR increases, the optical density increases. EOAR showed important antioxidant effect with effective concentration EC-50 = 64.60 ± 3.71 µg/mL compared to BHT and quercetin that showed EC-50 value of 91.07 ± 5.18 µg/mL and 71.55 ± 4.81 µg/mL (Figure 2D), respectively. Our results corroborate those already found by Banslama et al. EOs from the same species harvested in Algeria recorded an EC-50 value of 67 µg/mL [47]. However, a study conducted by Chebbac on the evaluation of the antioxidant power of essential oils of *Artemisia aragonensis*, revealed that the EC-50 was around 0.118 ± 0.008 mg/mL. Thus, the effect of EOAR was less important but it remains closer to *Artemisia aragonensis* [48].

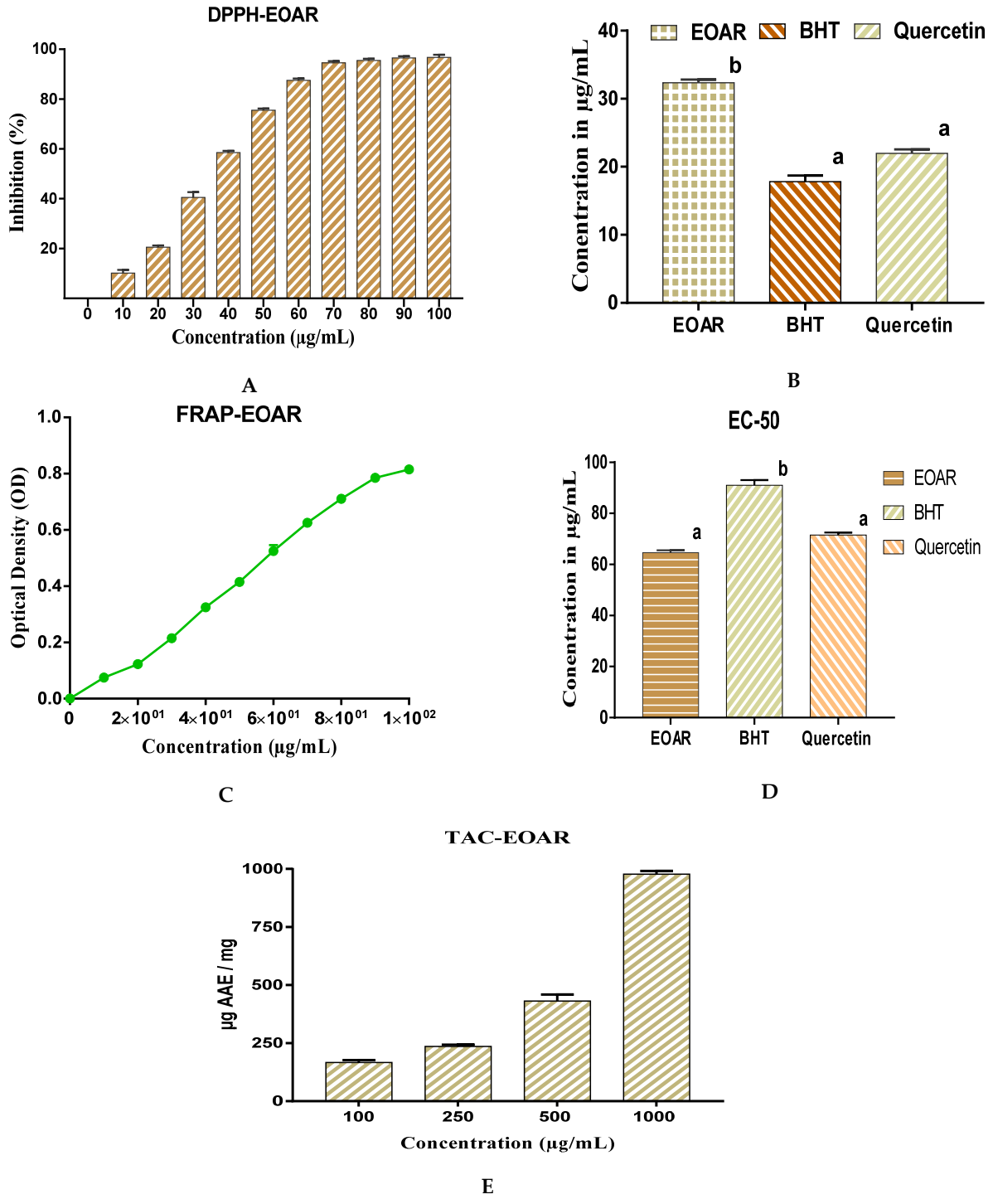

Figure 2. Antioxidant power of EOAR. Free radical inhibition as a function of EOAR concentration by use of DPPH test (**A**); IC50 value of DPPH test (**B**); Optical density as a function of EOAR concentration by use of FRAP test (**C**); effective concentration (EC50) of EAOR by use of FRAP test (**D**); Total antioxidant capacity of EOAR by use of phosphomolybdate method (TAC) (**E**). (results are represented as Means ± SD; n = 3. Columns marked with the same letter do not represent a significant difference).

The total antioxidant capacity of EOAR was evaluated at various concentrations (100, 250, 500, and 1000 µg/mL). The phosphomolybdenum method is established through the reduction of Mo (VI) to Mo (V) due to the antioxidant activity. This leads to complex formation with maximal absorption at 695 nm. As presented in Figure 2E, important total

antioxidant capacity was observed in EOAR in a dose-dependent manner. The antioxidant capacity was determined to be 167.24 ± 12.35 µg AAE/mg for 100 µg/mL EOAR as the lowest concentration used for testing, while 977.51 ± 22.38 µg AAE/mg was recorded for the highest concentration (1000 µg/mL EOAR) (Figure 2E). By use of phosphomolybdate method, *Lavandula dentata* essential oils revealed antioxidant capacity of 1.28 ± 2.28 µg AAE/mg for 1000 µg/mL EO [35], while EOs of *Withania frutescens* exhibited antioxidant power of 3.78 ± 0.41 µg AAE/mg for 1000 µg/mL EO [34]. Our results reveal definitely that EOARs have good total antioxidant power compared to some other essential oils with prominent therapeutic and pharmacological power.

Table 1. Compounds identified in EOARs by GC/MS.

Peaks	Retention Time	Compound	Chemical Class	Kovats Index	Area (%)
1	10.580	O-Cymene	MOH	1026	7.06
2	11.607	Y-Terpinene	MOH	1056	2.05
3	18.330	Camphor	MOO	1290	21.41
4	18.550	Carvacrol	MOO	1299	2.54
5	22.707	α-Himachalene	SEH	1451	15.88
6	23.412	Longifolene	SEH	1407	9.77
7	23.522	α-Cedrene	SEH	1411	1.42
8	23.951	α-Cuprenene	SEH	1505	33.48
9	24.144	Isolongifolene	SEH	1390	1.82
10	24.341	α-Cadinen	SEH	1538	1.15
11	24.550	Y-Dehydro-ar himachalene	SEH	1517	2.31
12	27.069	β-Himachalene oxide	SEO	1616	1.09
		Monoterpene hydrocarbon (MOH)			9.11%
		Monoterpene oxygene (MOO)			23.95%
		Sesquiterpene hydrocarbon (SEH)			65.83
		Sesquiterpene oxygene (SEO)			1.09%
		Total			99.98%

Altogether, the evaluation of the antioxidant power of EOAR by DPPH, FRAP, and TAC methods revealed the antioxidant potential of this essential oil to be potent. This pharmacological effect may be due to the richness of EOAR in various bioactive compounds (Table 1). The presented results here agreed with the literature since essential oils rich in carvacrol, thymol, o-cymene, and γ-Terpinene possessed potent antioxidant power [30,31,34,35,41,45].

3.3. Antimicrobial Power of EOAR

Results showed that all tested microbial isolates were resistant to the positive control Kanamycine (Figures 3 and 4). However, the EOAR showed antibacterial activity with varying magnitudes. The diameter of inhibition zone of EOAR varied from 23.50 ± 2.31 mm to 29.50 ± 2.21 mm respectively for *B. subtilis* and *E. coli* (Figure 3A). These results were confirmed by studies carried out by El Hanbali and co-workers, who found that the inhibition diameter of *E. coli* and *S. aureus* was of the order of 24 mm and 36 mm respectively [41]. In addition, *A. radiata* extracts showed potent antibacterial activity against *B. subtilis*, with a zone of inhibition ranging from 18 to 23 mm for the methanolic extract and from 12 to 18 mm for the aqueous extract [47]. The microdilution method revealed that the MIC was of the order of 12.71 ± 1.59 µg/mL for *E. coli* and 23.53 ± 0.78 µg/mL for *S. aureus* (Figure 3B). Recent studies on the crude extracts showed that the methanolic extract of *A. radiata* had a better MIC against *S. aureus* and *B. subtilis* which was of the order of 6.25 µg/mL and 25 µg/mL, respectively [47]. Previous studies dealing with antimicrobial activity showed that EOs have a broad spectrum vs. various strains [30,31,34,36,44,46,48]. The antibacterial effect is exemplified by carvacrol, terpinene, o-cymene, camphor, Longifolene, and Cedrene as responsible compounds for the antimicrobial activity of EOs. These volatile chemicals are known as "green antimicrobials"

because of their low cost, biocompatibility, antibacterial activity, ability to reverse resistance, and low toxicity to eukaryotic cells [49].

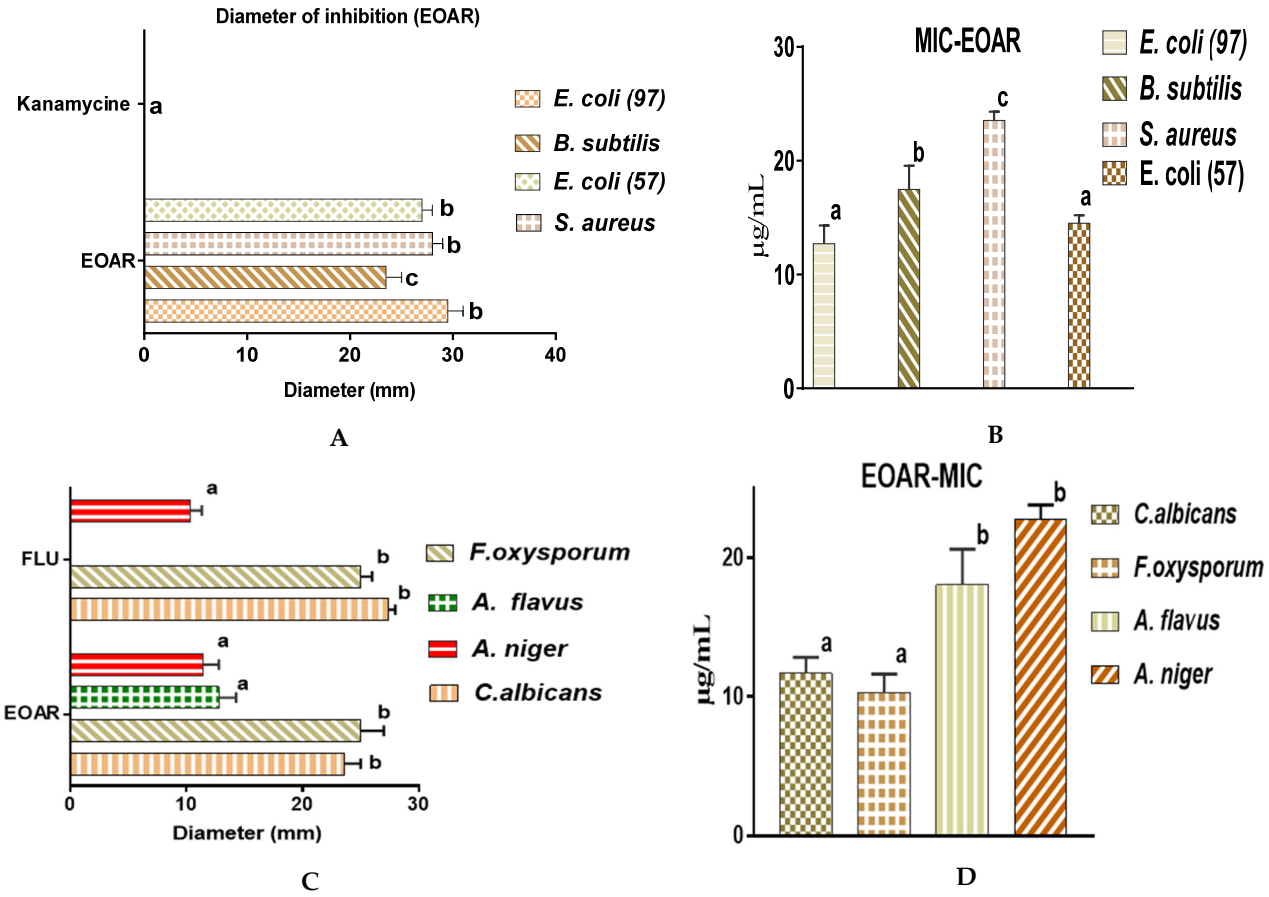

Figure 3. Antibacterial power of EOAR by use of disc method (**A**) and minimal inhibitory concentration (**B**). Antifungal potency of EOAR by use of disc (**C**) and microdilution assays (**D**). Columns with the same letters do not represent a significant difference, (Means ± SD; $n = 3$).

The antifungal potency of EOAR by disc method revealed that the largest inhibition diameter was of the order of 25.12 ± 2.82 mm observed on *F. oxysporum* and the smallest diameter was 11.42 ± 1.90 mm observed on *A. niger*. On the other hand, *A. flavus* showed resistance against the positive control (Fluconazole) (Figure 3C). The microdilution method revealed that the lowest MIC was recorded for *F. oxysporum* (10.31 ± 1.34 µg/mL), while the highest MIC was recorded for *A. niger* (22.75 ± 1.06 µg/mL) (Figure 3D).

These results were confirmed by studies performed on *Artemisia aragonensis* essential oils such that the MIC was in the order of 21.50 µg/mL for *F. oxysporum* and *A. niger*. The antifungal efficacy of EOAR remains less important than those found on *Nigella sativa* essential oils as the lowest MIC was of the order of 0.67 µg/mL recorded on *C. albicans* and the highest MIC was of the order of 2.69 µg/mL [36]. The antifungal effectiveness of EOAR is due to its richness in volatile compounds, as most studies have shown that carvacrol, thyme camphor, and o-cymene were inhibitors of the growth of phytopathogenic strains [31,36,46]. Several studies have been devoted to the use of various bioactive compounds, either natural or synthetic, to suppress pathogenic and phytopathogenic fungi in general, and *A. niger*, *A. flavus*, *F. oxysporum*, and *C. albicans* in particular [35,46].

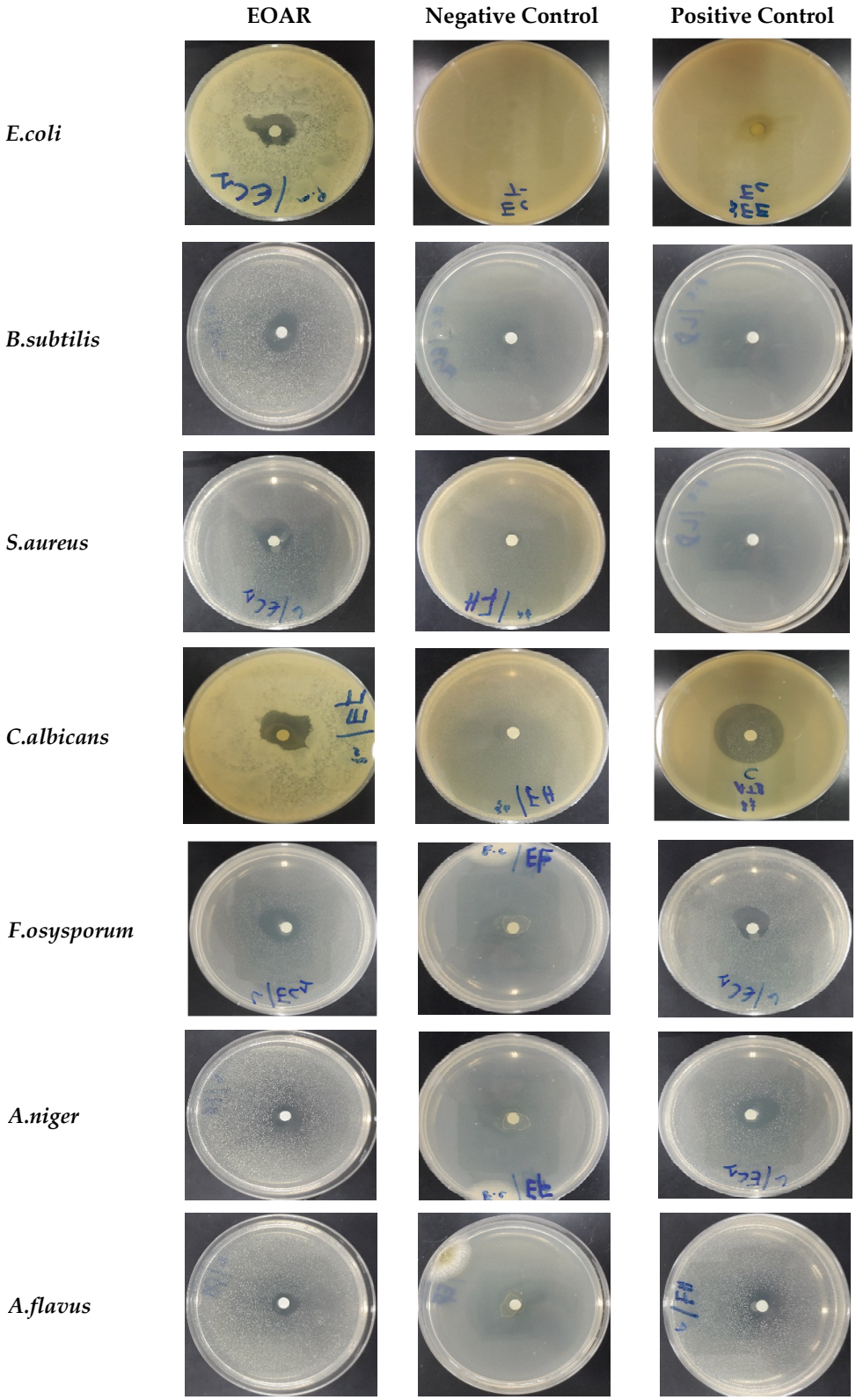

Figure 4. Antimicrobial potency of EOAR by use of the disc method. Positive control was Fluconazole (antifungal pontentiality) and Kanamycin (antibacterial pontentiality).

The variety and importance of terpene chemicals found in EOAR may be behind the observed activities. It is generally recognized that a complex mixture of volatile compounds could potentially elicit various biological responses, although the exact mechanism of action of EOs is still elusive. Over the years, numerous studies on the antibacterial activity of

EOs have been conducted [17]. Recent research has started to more fully understand how EOs and their main constituents interact with microbial targets [17,21]. The mechanisms of action are complicated due to the chemical diversity of EOs, which allows for a variety of compounds that can individually act on several targets [50]. Additionally, EOs antimicrobial mechanisms have been discussed in literature, which showed that Eos could work against morphological and regulatory levels of microbes [17,44]. Due to their distinct hydrophobic nature, EOs can simply act on the cell's outer membrane and cytoplasm, which may cause a disruption of the bacterial structures and an increase in permeability [17,30,51]. The major mechanisms of action of EOs and related compounds can interfere with the maintenance of the cell's energy status, the solute transport and membrane coupled energy transduction processes, and metabolic control by acting on the cell membrane. By blocking autoinducer mediators, EOs can occasionally change the expression of operons [17,30,44].

3.4. Molecular Docking

In stimulated endothelial cells, NADPH oxidase is a major enzymatic source of oxygen free radicals [47]. Moreover, the inhibition of this protein represents a major key in the protection of cells against free radicals. Several in vitro studies have shown the antioxidant activity of *Anvillea radiata* [8,47,52]. In silico study carried out in this work showed the inhibitory effect of EOAR against NADPH oxidase expressed in free binding energy.

γ-dehydro-ar-himachalene, carvacrol, α-cadinene, γ-terpinene, and β-himachalene oxide were the most active compounds against the active site of NADPH oxidase with a glide score of −6.233, −6.082, −5.057, −4.91, and −4.415 Kcal/mol respectively. In addition, the other docked molecules showed energy between −4.121 and −2.759 kcal/mol. Results from docking runs are summarized in Table 2. Our results are in agreement with a study where carvacrol showed remarkable antioxidant activity according to 1,1-diphenyl-2-picrylhydrazyl assay and Rancimat assay [53]. 2D and 3D viewers of *Anvillea radiata* essential oils docked in the active site of NADPH oxidase showed the formation of two hydrogen bond between the GLY 161 and CYS 242 residue and the OH group of carvacrol (Figures 5 and 6).

Table 2. Docking results with essential oil of *Anvillea radiata* in active site of NADPH (PDB: 2CDU).

	Glide Gscore	Glide Emodel	Glide Energy
γ-dehydro-ar-himachalene	−6.233	−31.939	−22.777
Carvacrol	−6.082	−32.356	−23.536
α-cadinene	−5.057	−27.232	−21.43
γ-terpinene	−4.91	−24.19	−19.057
β-himachalene oxide	−4.415	−17.108	−15.532
O-cymen	−4.121	−35.819	−29.056
Longifolene	−3.861	−29.233	−23.893
α-himachalene	−3.859	−19.536	−11.804
Camphor	−3.845	−21.596	−17.537
α-cedrene	−3.841	−9.574	−0.657
β-himachalene oxide	−3.752	−19.554	−15.836
Longifolene	−3.097	−12.851	−10.753
Isolongifolene	−2.759	−13.925	−4.531

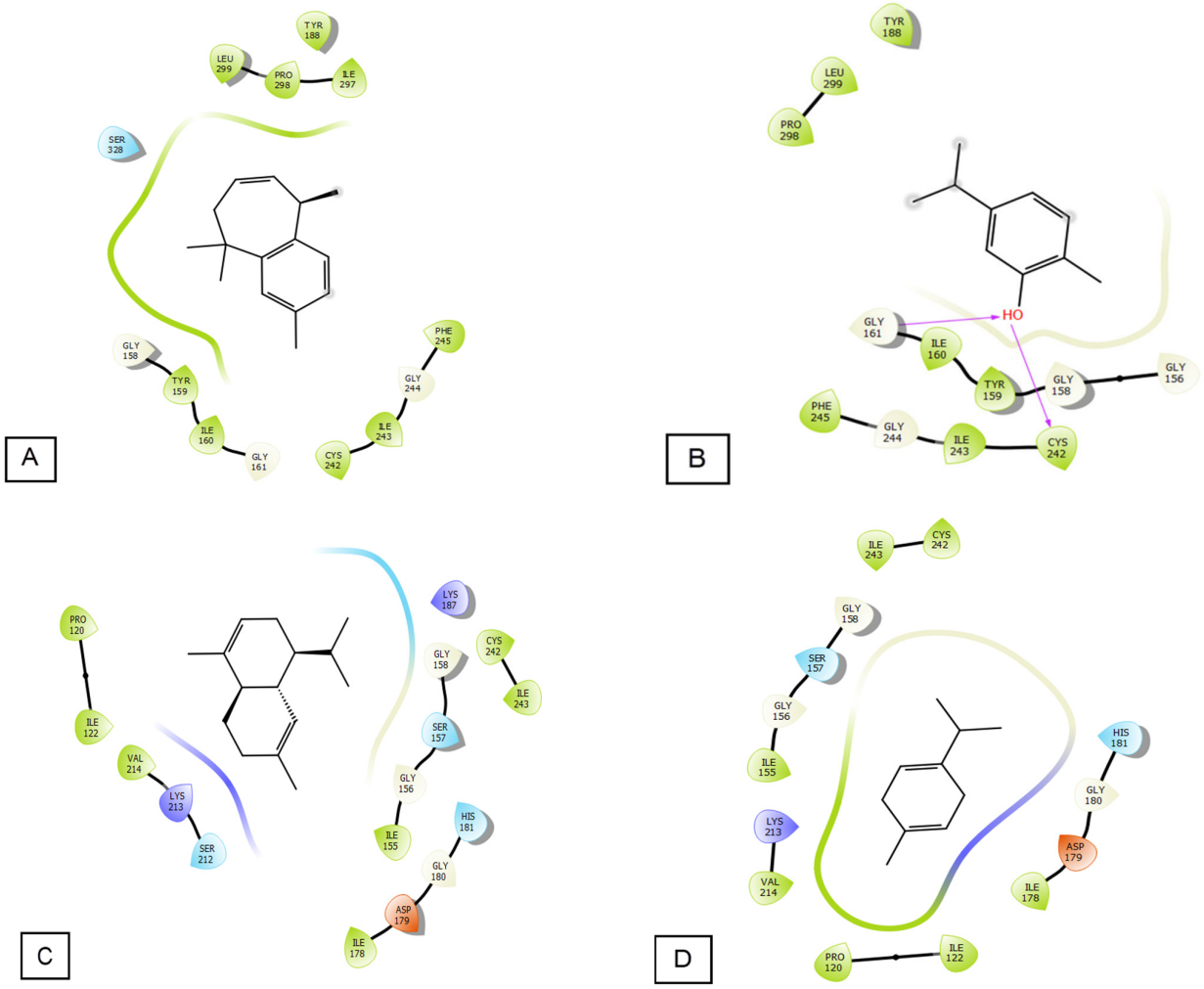

Figure 5. 2D diagrams of ligands interactions with the active site of NADPH. (**A**): γ-dehydro-arhimachalene; (**B**): Carvacrol; (**C**): α-cadinene; (**D**): γ-terpinene.

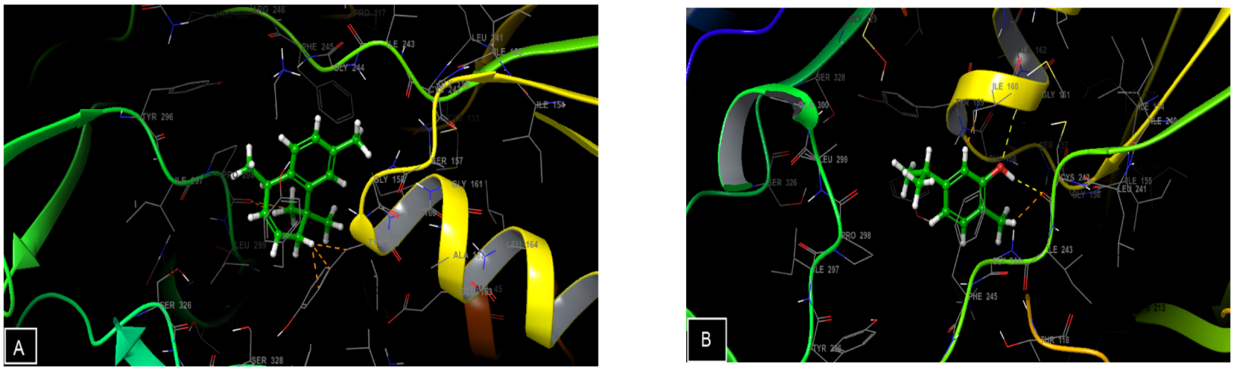

Figure 6. *Cont.*

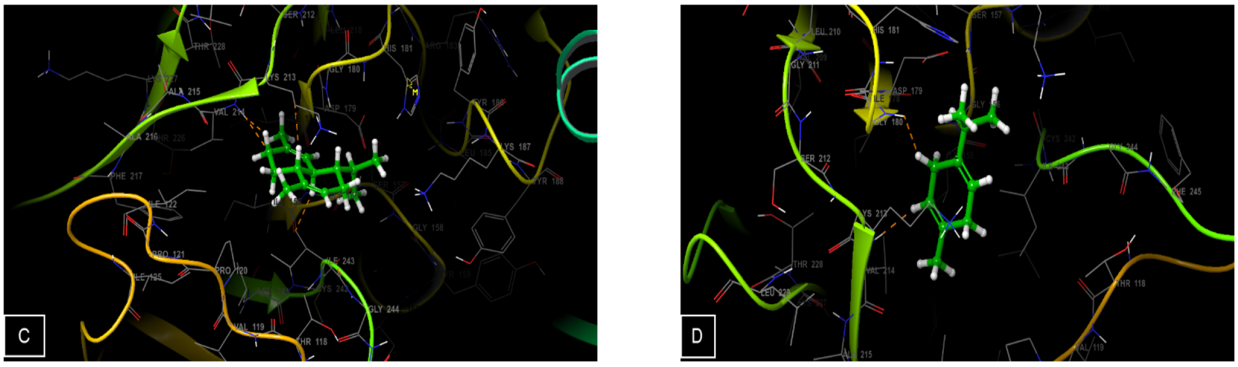

Figure 6. 3D diagrams of ligands interactions with the active site of NADPH. (**A**): γ-dehydro-arhimachalene; (**B**): Carvacrol; (**C**): α-cadinene; (**D**): γ-terpinene.

4. Conclusions

In conclusion, against clinically significant and drug-resistant bacteria, *A. radiata* demonstrated outstanding antioxidant and antimicrobial properties. These results are noteworthy because they may also involve potential use of EOAR as an alternative treatment instead of conventional antimicrobial therapies. Further works dealing with potential toxicities of EOAR on non-target organisms and humans are much appreciated before any medical application.

Author Contributions: All authors contributed to writing of original draft, formal analysis, reviewing and editing, data curation, validation. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by the Researchers Supporting Project, King Saud University, Riyadh, Saudi Arabia for funding this work through the project number (RSP2022R457).

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to extend their sincere appreciation to the Researchers Supporting Project, King Saud University, Riyadh, Saudi Arabia for funding this work through the project number (RSP2022R457).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rollinger, B.J.M.; Stuppner, H.; Hofbauergasse, C.M. Virtual screening for the discovery of bioactive natural products. In *Natural Compounds in Drugs Volume I*; Springer: Berlin/Heidelberg, Germany, 2008; Volume 65, pp. 211–249.
2. Mahomoodally, M.F. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. *Evid.-Based Complementary Altern. Med.* **2013**, *2013*, 617459. [CrossRef]
3. Jamshidi-Kia, F.; Lorigooini, Z.; Amini-Khoei, H. Medicinal plants: Past history and future perspective. *J. Herbmed Pharmacol.* **2018**, *7*, 1–7. [CrossRef]
4. Ghanmi, M.; Aafi, A.; Satrani, B.; Aberchane, M. Aromatic and Medicinal Plants in North Africa: Opportunities, Constraints and Prospects. In *Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics*; Wiler Online Library: Hoboken, NJ, USA, 2014; pp. 411–423.
5. Idm'Hand, E.; Msanda, F.; Cherifi, K. Ethnobotanical study and biodiversity of medicinal plants used in the Tarfaya Province, Morocco. *Acta Ecol. Sin.* **2020**, *40*, 134–144. [CrossRef]
6. Perveen, S.; Al-Taweel, A.M.; Yusufoglu, H.S.; Fawzy, G.A.; Foudah, A.; Abdel-Kader, M.S. Hepatoprotective and cytotoxic activities of Anvillea garcinii and isolation of four new secondary metabolites. *J. Nat. Med.* **2017**, *72*, 106–117. [CrossRef]
7. Hebi, M.; Eddouks, M. Glucose Lowering Activity of Anvillea Radiata Coss & Durieu in Diabetic Rats. *Cardiovasc. Haematol. Disord.-Drug Targets* **2018**, *18*, 71–80. [CrossRef]
8. Kandouli, C.; Cassien, M.; Mercier, A.; Delehedde, C.; Ricquebourg, E.; Stocker, P.; Mekaouche, M.; Leulmi, Z.; Mechakra, A.; Thétiot-Laurent, S.; et al. Antidiabetic, antioxidant and anti inflammatory properties of water and n-butanol soluble extracts from Saharian Anvillea radiata in high-fat-diet fed mice. *J. Ethnopharmacol.* **2017**, *207*, 251–267. [CrossRef]
9. Akdad, M.; Ajebli, M.; Breuer, A.; Khallouki, F.; Owen, R.W.; Eddouks, M. Study of Antihypertensive Activity of Anvillea radiata in L-Name-Induced Hypertensive Rats and HPLC-ESI-MS Analysis. *Endocr. Metab. Immune Disord. Drug Targets* **2020**, *20*, 1059–1072. [CrossRef]

10. Gutteridge, J.C.M.; Halliwell, B. Free Radicals and Antioxidants in the Year 2000: A Historical Look to the Future. *Ann. N. Y. Acad. Sci.* **2006**, *899*, 136–147. [CrossRef]
11. Devasagayam, T.P.A.; Tilak, J.C.; Boloor, K.K.; Sane, K.S. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects R ESEARCH. *Japi* **2004**, *52*, 4.
12. Barber, D.A.; Harris, S.R. Oxygen Free Radicals and Antioxidants: A Review. *Am. Pharm.* **1994**, *34*, 26–35. [CrossRef]
13. Bandyopadhyay, U.; Das, D.; Banerjee, R.K. Reactive oxygen species: Oxidative da and pathogenesis. *Curr. Sci.* **1999**, *77*, 658–666.
14. Nath, K.A.; Norby, S.M. Reactive Oxygen Species and Acute Renal Failure of reactive oxygen species. *Am. J. Med.* **2000**, *109*, 665–678. [CrossRef]
15. Wolff, S.P.; Garner, A.; Dean, R.T. Free radicals, lipids and protein degradation. *Trends Biochem. Sci.* **1986**, *11*, 27–31. [CrossRef]
16. Hensley, K.; Floyd, R.A. Reactive Oxygen Species and Protein Oxidation in Aging: A Look Back, A Look Ahead. *Arch. Biochem. Biophys.* **2002**, *397*, 377–383. [CrossRef]
17. Bouyahya, A.; Bakri, Y.; Et-Touys, A.; Talbaoui, A.; Khouchlaa, A.; Charfi, S.; Abrini, J.; Dakka, N. Résistance aux antibiotiques et mécanismes d'action des huiles essentielles contre les bactériesResistance to antibiotics and mechanisms of action of essential oils against bacteria. *Phytothérapie* **2017**, 1–11. [CrossRef]
18. Chaudhary, A.S. A review of global initiatives to fight antibiotic resistance and recent antibiotics discovery. *Acta Pharm. Sin. B* **2016**, *6*, 552–556. [CrossRef] [PubMed]
19. Dung, N.T.; Kim, J.M.; Kang, S.C. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. *Food Chem. Toxicol.* **2008**, *46*, 3632–3639. [CrossRef]
20. Et-Touys, A.; Fellah, H.; Mniouil, M.; Bouyahya, A.; Dakka, N.; Abdennebi, E.H.; Sadak, A.; Bakri, Y. Screening of Antioxidant, Antibacterial and Antileishmanial Activities of *Salvia officinalis* L. Extracts from Morocco. *Br. Microbiol. Res. J.* **2016**, *16*, 1–10. [CrossRef]
21. Bouyahya, A.; Abrini, J.; El-Baabou, A.; Dakka, Y.B.A.N. Determination of Phenol Content and Antibacterial Activity of Five Medicinal Plants Ethanolic Extracts from North-West of Morocco. *J. Plant Pathol. Microbiol.* **2016**, *7*, 2. [CrossRef]
22. Bouyahya, A.; El Moussaoui, N.; Abrini, J.; Bakri, Y.; Dakka, N. Determination of Phenolic Contents, Antioxidant and Antibacterial Activities of Strawberry Tree (*Arbutus unedo* L.) Leaf Extracts. *Br. Biotechnol. J.* **2016**, *14*, 1–10. [CrossRef]
23. Rehman, R.; Hanif, M.A.; Mushtaq, Z.; Al-Sadi, A. Biosynthesis of essential oils in aromatic plants: A review. *Food Rev. Int.* **2015**, *32*, 117–160. [CrossRef]
24. Petrovi, J.; Stojkovi, D.; Sokovi, M. *Terpene Core in Selected Aromatic and Edible Plants: Natural Health Improving Agents*, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2019.
25. Tongnuanchan, P.; Benjakul, S. Essential Oils: Extraction, Bioactivities, and Their Uses for Food Preservation. *J. Food Sci.* **2014**, *79*, R1231–R1249. [CrossRef]
26. Falleh, H.; Ben Jemaa, M.; Saada, M.; Ksouri, R. Essential oils: A promising eco-friendly food preservative. *Food Chem.* **2020**, *330*, 127268. [CrossRef]
27. Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines* **2016**, *3*, 25. [CrossRef]
28. Kalemba, D.; Kunicka, A. Antibacterial and Antifungal Properties of Essential Oils. *Curr. Med. Chem.* **2003**, *10*, 813–829. [CrossRef]
29. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
30. EL Moussaoui, A.; Bourhia, M.; Jawhari, F.; Salamatullah, A.; Ullah, R.; Bari, A.; Mahmood, H.M.; Sohaib, M.; Serhii, B.; Rozhenko, A.; et al. Chemical Profiling, Antioxidant, and Antimicrobial Activity against Drug-Resistant Microbes of Essential Oil from *Withania frutescens* L. *Appl. Sci.* **2021**, *11*, 5168. [CrossRef]
31. Chebbac, K.; EL Moussaoui, A.; Bourhia, M.; Salamatullah, A.M.; Alzahrani, A.; Guemmouh, R. Chemical Analysis and Antioxidant and Antimicrobial Activity of Essential oils from *Artemisia negrei* L. against Drug-Resistant Microbes. *Evid. Based Complement. Altern. Med.* **2021**, *2021*, 1–9. [CrossRef]
32. Tepe, B.; Daferera, D.; Sokmen, A.; Sokmen, M.; Polissiou, M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (*Lamiaceae*). *Food Chem.* **2005**, *90*, 333–340. [CrossRef]
33. El Moussaoui, A.; Jawhari, F.Z.; Almehdi, A.M.; Elmsellem, H.; Benbrahim, K.F.; Bousta, D.; Bari, A. Antibacterial, antifungal and antioxidant activity of total polyphenols of *Withania frutescens* L. *Bioorganic Chem.* **2019**, *93*, 103337. [CrossRef]
34. El Moussaoui, A.; Kadiri, M.; Bourhia, M.; Agour, A.; Salamatullah, A.M.; Alzahrani, A.; Alyahya, H.K.; Albadr, N.A.; Chedadi, M.; Sfaira, M.; et al. Promising Antioxidant and Anticorrosion Activities of Mild Steel in 1.0 M Hydrochloric Acid Solution by *Withania frutescens* L. Essential Oil. *Front. Chem.* **2021**, *9*, 760. [CrossRef]
35. El Abdali, Y.; Agour, A.; Allali, A.; Bourhia, M.; El Moussaoui, A.; Eloutassi, N.; Salamatullah, A.M.; Alzahrani, A.; Ouahmane, L.; Aboul-Soud, M.A.M.; et al. *Lavandula dentata* L.: Phytochemical Analysis, Antioxidant, Antifungal and Insecticidal Activities of Its Essential Oil. *Plants* **2022**, *11*, 311. [CrossRef]
36. Zouirech, O.; Alyousef, A.A.; El Barnossi, A.; El Moussaoui, A.; Bourhia, M.; Salamatullah, A.M.; Ouahmane, L.; Giesy, J.P.; Aboul-Soud, M.A.M.; Lyoussi, B.; et al. Phytochemical Analysis and Antioxidant, Antibacterial, and Antifungal Effects of Essential Oil of Black Caraway (*Nigella sativa* L.) Seeds against Drug-Resistant Clinically Pathogenic Microorganisms. *BioMed Res. Int.* **2022**, *2022*, 1–11. [CrossRef]

37. Saghrouchni, H.; El Barnossi, A.; Chefchaou, H.; Mzabi, A.; Tanghort, M.; Remmal, A.; Fouzia, C. Study the effect of carvacrol, eugenol and thymol on fusariums sp responsible for lolium perenne fusariosis. *Ecol. Environ. Conserv.* **2020**, *26*, 1059–1067.
38. Remmal, A.; Bouchikhi, T.; Rhayour, K.; Ettayebi, M.; Tantaoui-Elaraki, A. Improved Method for the Determination of Antimicrobial Activity of Essential Oils in Agar Medium. *J. Essent. Oil Res.* **1993**, *5*, 179–184. [CrossRef]
39. Saghrouchni, H.; El Barnossi, A.; Salamatullah, A.; Bourhia, M.; Alzahrani, A.; Alkaltham, M.; Alyahya, H.; Tahiri, N.; Imtara, H.; Var, I. Carvacrol: A Promising Environmentally Friendly Agent to Fight Seeds Damping-Off Diseases Induced by Fungal Species. *Agronomy* **2021**, *11*, 985. [CrossRef]
40. Chalkha, M.; El Moussaoui, A.; Ben Hadda, T.; Berredjem, M.; Bouzina, A.; Almalki, F.A.; Saghrouchni, H.; Bakhouch, M.; Saadi, M.; El Ammari, L.; et al. Crystallographic study, biological evaluation and DFT/POM/Docking analyses of pyrazole linked amide conjugates: Identification of antimicrobial and antitumor pharmacophore sites. *J. Mol. Struct.* **2021**, *1252*, 131818. [CrossRef]
41. El Hanbali, F.; El Hakmaoui, A.; Mellouki, F.; El Rhaffari, L.; Akssira, M. Chemical Composition and Antibacterial Activity of Essential Oil of *Anvillea radiata* Coss. & Dur. *Nat. Prod. Commun.* **2007**, *2*, 595–597. [CrossRef]
42. Agati, G.; Brunetti, C.; Di Ferdinando, M.; Ferrini, F.; Pollastri, S.; Tattini, M. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiol. Biochem.* **2013**, *72*, 35–45. [CrossRef]
43. El Moussaoui, A.; Bourhia, M.; Jawhari, F.Z.; Khalis, H.; Chedadi, M.; Agour, A.; Salamatullah, A.M.; Alzahrani, A.; Alyahya, H.K.; Alotaibi, A.; et al. Responses of *Withania frutescens* (L.) Pauquy (Solanaceae) Growing in the Mediterranean Area to Changes in the Environmental Conditions: An Approach of Adaptation. *Front. Ecol. Evol.* **2021**, *9*, 666005. [CrossRef]
44. Yu, Z.; Tang, J.; Khare, T.; Kumar, V. The Alarming Antimicrobial Resistance in ESKAPEE Pathogens: Can Essential Oils Come tothe Rescue? *Fitoterapia* **2020**, *140*, 10443. [CrossRef] [PubMed]
45. Mssillou, I.; Agour, A.; Allali, A.; Saghrouchni, H.; Bourhia, M.; El Moussaoui, A.; Salamatullah, A.M.; Alzahrani, A.; Aboul-Soud, M.A.M.; Giesy, J.P.; et al. Antioxidant, Antimicrobial, and Insecticidal Properties of a Chemically Characterized Essential Oil from the Leaves of *Dittrichia viscosa* L. *Molecules* **2022**, *27*, 2282. [CrossRef] [PubMed]
46. Lafraxo, S.; El Barnossi, A.; El Moussaoui, A.; Bourhia, M.; Salamatullah, A.M.; Alzahrani, A.; Akka, A.A.; Choubbane, A.; Akhazzane, M.; Aboul-Soud, M.A.M.; et al. Essential Oils from Leaves of *Juniperus thurifera* L., Exhibiting Antioxidant, Antifungal and Antibacterial Activities against Antibiotic-Resistant Microbes. *Horticulturae* **2022**, *8*, 321. [CrossRef]
47. Benslama, A.; Harrar, A.; Gül, F.; Demirtaş, I. In vitro Antioxidant, Antibacterial Activities and HPLC-TOF/MS Analysis of *Anvillea radiata* (Asteraceae) Extracts. *Curr. Nutr. Food Sci.* **2019**, *15*, 376–383. [CrossRef]
48. Chebbac, K.; Ghneim, H.K.; El Moussaoui, A.; Bourhia, M.; El Barnossi, A.; Ouaritini, Z.B.; Salamatullah, A.M.; Alzahrani, A.; Aboul-soud, M.A.M.; Giesy, J.P.; et al. Antioxidant and Antimicrobial Activities of Chemically-Characterized Essential Oil from Artemisia. *Molecules* **2022**, *27*, 1136. [CrossRef]
49. Lu, M.; Dai, T.; Murray, C.K.; Wu, M.X. Bactericidal Property of Oregano Oil against Multidrug-Resistant Clinical Isolates. *Front. Microbiol.* **2018**, *9*, 2329. [CrossRef]
50. Tiwari, B.K.; Valdramidis, V.; Donnell, C.P.O.; Muthukumarappan, K.; Bourke, P.; Cullen, P.J. Application of Natural Antimicrobials for Food Preservation. *J. Agric. Food Chem.* **2009**, *57*, 5987–6000. [CrossRef]
51. Bouhdid, S.; Abrini, J.; Amensour, M.; Zhiri, A.; Espuny, M.J.; Manresa, A. Functional and ultrastructural changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Cinnamomum verum essential oil. *J. Appl. Microbiol.* **2010**, *109*, 1139–1149. [CrossRef]
52. Hamada, D.; Ladjel, S. Chemical Composition, In-Vitro Anti-microbial and Antioxidant Activities of the Methanolic Extract of *Anvillea radiata* Asteraceae. *Res. J. Pharm. Biol. Chem.* **2015**, *6*, 1367.
53. Mastelić, J.; Jerković, I.; Blažević, I.; Poljak-Blaži, M.; Borović, S.; Ivančić-Baće, I.; Smrečki, V.; Žarković, N.; Brčić-Kostic, K.; Vikić-Topić, D.; et al. Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives. *J. Agric. Food Chem.* **2008**, *56*, 3989–3996. [CrossRef] [PubMed]