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Gas Chromatography–Mass Spectrometry Chemical Profiling and Radical Scavenging Potential of Sesquiterpene-Rich Essential Oil of *Polygonum equisetiformme* Sm.: In Silico Study on Hematopoietic Cell Kinase (Hck) and Human Peroxiredoxin 5

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Abstract: Essential oils (EOs) are advised by traditional medical systems for the treatment of a variety of disorders worldwide. In many ancient medical systems around the world, *Polygonum* herbs have been employed as remedies including *P. equisetiforme* Sm. The EO profile of *P. equisetiforme* and its bioactivities have yet to be discussed in depth. As a result, the current study aims to investigate the chemical profile and free radical scavenging capacity of *P. equisetiforme* EO. Hydrodistillation was used to obtain the EO from *P. equisetiforme*, and gas chromatography–mass spectrometry (GC-MS) was used for analysis. A total of forty-three compounds, including terpenes and sesquiterpenes as the main components (76.13% and 69.06%, respectively), were identified in the oil using the GC-MS analysis. The main constituents of the oil were hexahydrofarnesyl acetone (29.45%), 7-epi-selinene (14.45%), isospathulenol (8.35%), and *n*-docosane (6.79%). The chemosystematic significance of the plant was established via multivariate assessing, comprising principal component analysis (PCA), hierarchical clustering, and constellation plot, of the EO principal components of the various *Polygonum* plants. The *P. equisetiforme* exhibited different associations with the studied *Polygonum* spp. Then, the scavenging of the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used to evaluate the radical scavenging abilities of EO compared with those of vitamin C, a reference antioxidant. *P. equisetiforme* EO exhibited the scavenging capacity of the DPPH and the ABTS free radical with respective IC_{50} values of 470.01 and 113.74 mg L^{-1} compared with vitamin C, and with IC_{50} values of 39.06 and 26.09 mg L^{-1}, respectively. The in silico studies revealed that the oxygenated sesquiterpenes, especially ar-turmerone, hexahydrofarnesyl acetone, and *5E*,*9E*-farnesyl acetone, exhibited the best fitting with hematopoietic cell kinase (Hck) and human Peroxiredoxin 5 proteins with ΔG values of −6.14 and −4.93, −6.83 and −5.34, and −7.08 and −5.47 kcal/mol, respectively. The major components' combined or individual effects may be responsible for the antioxidant properties. Therefore, additional extensive studies are advised to characterize the essential compounds as radical scavenger agents, either individually or in combination.

Keywords: volatile components; chemical fingerprint; chemometric study; antioxidant; molecular docking

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1. Introduction

New therapeutic compounds can be found mostly in natural products and their derivatives [1]. Essential/volatile oils (EOs/VOs) are complex combinations of low-molecular-weight components that are extracted from plants using hydrodistillation, microwave, steam distillation, and solvent extraction [2]. The main components that give EOs their distinctive scent and biological effects are terpenes and phenylpropanoids [3,4]. Since the Middle Ages, EOs have been utilized extensively for fungicidal, bactericidal, virucidal, insecticidal, antiparasitic, medicinal, and cosmetic uses [5,6]. Traditional medical systems around the world recommend essential oils for a range of health issues [5]. They are frequently used as flavoring ingredients in foods, beverages, cosmetics, medications, and fragrances [5,6]. These days, they are particularly popular in the pharmaceutical, food industries, sanitary, and agricultural [6,7].

The polygonaceae family, including around 40 genera and 800 flowering plant species, is spread across tropical, subtropical, and temperate climates [8]. Around 150 plants belonging to the *Polygonum* genus are widely distributed in North Africa, Europe, and Western Asia [7,9]. Eight species of Egyptian flora have been identified as abundant, growing in Egypt's coastal and inland deserts, Nile Delta, and Sinai. Worldwide, *Polygonum* plants have been used for a number of traditional ailments, including the treatment of snakebite, heart illness, diarrhea, dermatitis, influenza, hemorrhoids, bacterial and skin infections, dysentery, and insomnia [10]. Additionally, the *Polygonum* plants are a significant supplier of numerous nutrient components, including proteins and lipids [11]. These plants are also potential sources of pharmaceutical and biological metabolites, mainly flavonoids, phenols, tannins, terpenes, stilbenes, anthraquinones, polysaccharides, and glycolipids [12]. In some countries, *P. equisetiforme* appears to be popular with domestic animals for grazing and browsing, providing significant economic benefits to the rural inhabitants [13]. *P. equisetiforme* is widely used in folk medicines for treating cold, cough, and sore throat [14]. It is additionally employed as a tea flavoring [15]. The previous chemical characterization of the *P. equisetiforme* revealed that this plant is very rich in phenolic acids, and flavonoids [16] with significant antioxidant, hepatoprotective, antibacterial, and antifungal effects [17,18].

Peroxiredoxins, a family of peroxidases, work in concert with other antioxidants, including enzymatic and non-enzymatic antioxidants, to control the concentrations of reactive oxygen species (ROS) and to safeguard cells from oxidative damage. Peroxiredoxin 5 (PRDX5), one of this group of enzymes, has been reported to reduce peroxides and hydroperoxides and prevent their intracellular accumulation [19]. The SRC family of cytoplasmic tyrosine kinases (SFKs) includes hematopoietic cell kinase (HCK), which is expressed in cells of the myeloid and B-lymphocyte cell lineages. Numerous kinds of leukemia are linked to excessive HCK activation, which promotes cell growth and survival by physical linkage with oncogenic fusion proteins and through functional interactions with receptor tyrosine kinases. Additionally, elevated HCK activity is shown in a variety of solid tumors, such as breast and colon cancer, and it is associated with lower patient survival rates. HCK promotes macrophage polarization towards a wound-healing, tumor-promoting, alternatively activated state, as well as promoting a growth factor and pro-inflammatory cytokine release from myeloid cells [20].

The EO profiling and bioactivities of *P. equisetiforme* have not been described before. Thus, the current work was intended to investigate the chemical profile of *P. equisetiforme* EO extracted from the aerial parts of this plant as well as its antioxidant potential. Moreover, molecular docking studies of the major compounds of EO were carried out on human Peroxiredoxin 5 and hematopoietic cell kinase (Hck).

2. Materials and Methods

2.1. Plant Collection, Authentication, and Preparation

The healthy, flowering aerial parts of *P. equisetiforme* were gathered from various populations growing within homogenous sandy habitat along the Mediterranean Sea Coast, near Baltim City (31°31′56.56″ N, 31°18′38.12″ E), during the flowering season in

May 2022 (Figure 1). In this context, three composite samples from different patches of *P. equisetiforme* were collected for further preparation and analysis. According to Boulos [21] and Tackholm [22], plant authentication was carried out. At Mansoura University in Egypt's Faculty of Science, a voucher specimen (Mans. 0161605004) was made and added to the herbarium. All the plant materials were carefully removed from the dust, dried for a short time at 25–28 °C in an open, shaded area, and then processed into powder using a grinder (IKA® MF 10 Basic Microfine Grinder Drive, Breisgau, Germany).

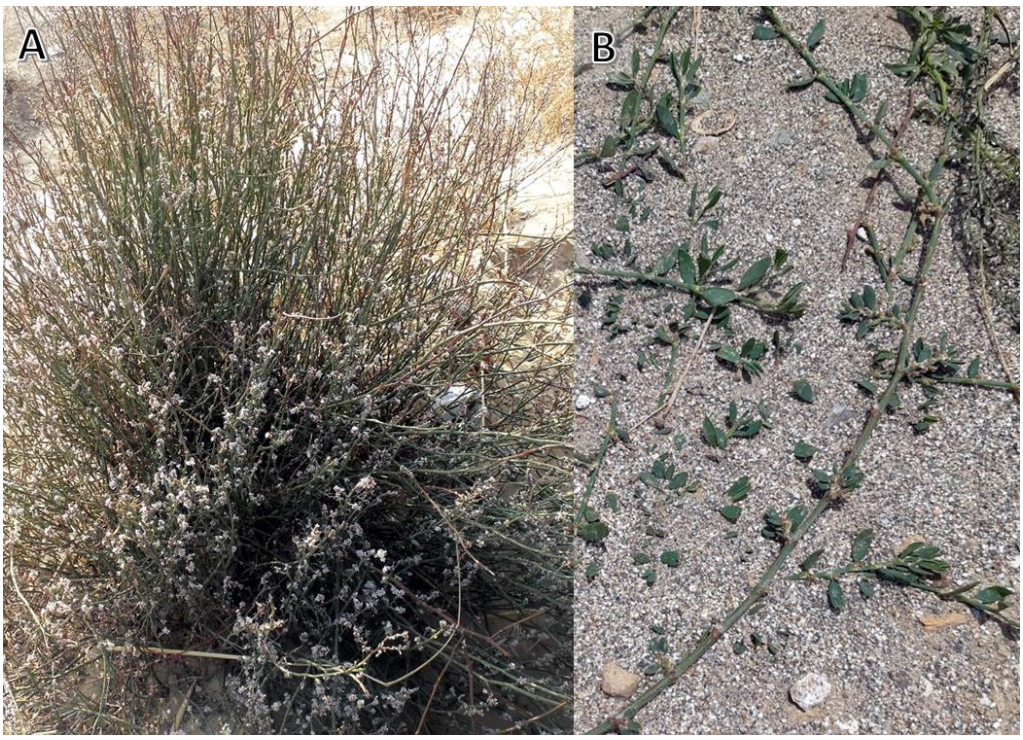

Figure 1. Photographs of *Polygonum equisetiforme* Sm.: (**A**) an overview of the mature plant; (**B**) a close view of young branches.

2.2. Chemical and Drugs

Ethanol (99%), *n*-hexane, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich, Darmstadt, Germany. The C_8-C_{22} *n*-alkane standards were bought from Sigma-Aldrich (St. Louis, MO, USA).

2.3. EO Extraction

The above-ground parts of *P. equisetiforme*s (150 g) were hydrodistilled for three hours using a Clevenger apparatus in a glass round flask (5 L) containing 2000 mL water. Next, the extracted EO layer was separated using 3 drops of *n*-hexane before being immediately dried with 0.5 g of anhydrous Na_2SO_4. The same procedure was used to obtain three EO samples from three plant samples (weighing 150 g each). For GC-MS analysis and biological evaluations, three distinctive dark-brown glass vials containing the three EO samples were put in a refrigerator set at 4 °C.

2.4. EO Analysis by Gas Chromatography–Mass Spectroscopy (GC-MS)

The chemical components of each of the three EO samples were identified using the same methods and under the same circumstances as those previously described by [23,24]. For the sake of summarizing, the GC-MS analysis used the TRACE Ultra-Gas Chromatography and Thermo Scientific ISQTM EC single quadrupole mass spectrometer, all of which are manufactured by THERMO ScientificTM Corporate, Waltham, MA, USA. The GC-MS

device was equipped with a TR-5 MS column that has an interior diameter of 30 m and a film that is 0.25 mm thick. With a flow rate of (1.0 mL min^{-1}) and a ratio of split of (1:10), helium was used as the carrier gas. The temperature schedule was set at 60 °C for a single minute and then increased to 240 °C at an average of 4.0 °C per minute. Each EO sample was injected in a modest volume (1 µL in hexane), at a 1:10 (v/v) concentration, into the injector and detector at 210 °C. The mass spectral data were collected at 70 eV using electron ionization (EI), with a spectrum spanning *m/z* 40–450. The chemical composition was determined by employing the AMDIS (automated mass spectral deconvolution and identification) applications, in addition to access to the Wiley Spectral Library collection and the NIST Library database (Gaithersburg, MD, USA; Wiley, Hoboken, NJ, USA), which were used for calculating of the retention indices relative to *n*-alkanes (C_8–C_{22}) or assessment to the mass spectral data of authentic components. The monoterpenes, camphene, d-limonene, pinene (α- and β-), linalool, geraniol, α-terpineol, and citral, and the sesquiterpenes, elemene (α- and β-), α-copaene, germacrene D, β-caryophyllene, cubedol, α-eudesmol, and spathulenol, were used as the references authenticated components in the GC-MS analysis. Upon completion of the GC-MS analysis, the three EO samples were gathered and stored in a single black glass vial pending the beginning of the biological investigations.

2.5. DPPH and ABTS Scavenging Activity

According to Miguel [25], the isolated EO from *P. equisetiforme* was tested for its antioxidant efficacy by measuring its ability to scavenge DPPH radicals. Using ethanol as the solvent, an array of EO concentrations of 65.5, 125, 250, 500, and 1000 mg L^{-1} were created. Equal amounts of freshly made DPPH (0.3 mM) and EO were created in test tubes, vigorously mixed, and incubated for 20 min in the dark at room temperature. Subsequently, the spectrophotometer (Analytik Jena, Jena, Germany) was used to determine the absorbance at 517 nm. In accordance with Re et al. [26], the antioxidant activity of *P. equisetiforme* EO via the reduction of ABTS was performed. In the DPPH assay, the EO concentrations range of 65.5–1000 mg L^{-1} were prepared in ethanol. In brief, 2 mL of newly made ABTS were combined with around 0.2 mL of each concentration, and the mixture was then incubated for 6 min in the dark. A Spectronic 21D spectrophotometer (Milton Roy, CA, USA) was applied to compute the color absorbance at 734 nm. Furthermore, a standard scavenger drug as vitamin C was created as a positive control within a range of concentrations of 12.5–125 mg L^{-1}. It was handled in the same way as the EO as previously mentioned. The following calculation was used to compute the ratio of scavenging efficiency in both methods.

$$\text{Scavenging efficiency \%} = 100 \times \left(1 - \frac{\text{A sample}}{\text{A control}} \right)$$

where A sample and A control = the recorded absorbances of the sample and control, respectively.

Additionally, several quantities of vitamin C (12.5–125 mg L^{-1}) were generated, and their antioxidant activity was assessed as previously stated for the EO. This was carried out to compare the effectiveness of the antioxidant with that of a standard antioxidant.

2.6. Data Treatments and Statistical Analysis

The data of free radical scavenging activity data based on three replications was treated with one-way ANOVA followed by Tukey's HSD test using the CoStat program, version 6.311 (CoHort Software, Monterey, CA, USA. For chemometric analysis, we prepared a dataset of 37 chemical compounds that were identified as major identified compounds with an abundance of more than 2.5% of the total EO mass. These compounds were identified as major compounds within 11 *Polygonum* species including our studied plant, *P. equisetiforme* as well as *P. odoratum*, *P. lapathifolium*, *P. arenarium*, *P. aviculare*, *P. arenastrum*, *P. bellardii*, *P. cognatum*, *P. persicaria*, *P. minus*, and *P. bistorta* subsp. *carneum*. The dataset was subjected to multivariate analysis, principal component analysis (PCA), and hierarchical cluster

analysis. These analyses were performed using the JMP® Pro 16.0.0 (SAS Institute Inc., Cary, NC, USA).

2.7. Molecular Docking Studies

Docking studies have been carried out using Molecular Operating Environment (MOE) software version 2015.10 [27]. The catalytic domains of human Peroxiredoxin 5 (PDB ID: 1HD2) [28] and hematopoietic cell kinase (Hck), one of Src family tyrosine kinase (PDB ID: 2HCK) [29], were obtained from the protein data bank. The crystal structures were cleaned of unwanted solvents, ligands, and cofactors, and the default MOE "QuickPrep" module settings were employed. The "Site Finder" option was then used to generate the probable binding pockets containing the crucial residues. For validation of the docking process, the co-crystallized ligands were re-docked in the active binding sites. The database file (.mdb) for the energy-minimized compounds was generated and docked in the ligand sites. The best conformers were studied in both 2D and 3D representations. S-scores with RMSD values $\leq$ 2 Å are inserted in the results section.

3. Results and Discussions

3.1. Chemical Profiling of P. equisetiforme EO

The hydrodistillation of *P. equisetiforme* materials yielded 0.8% (*v*/*w*) dark yellow oil with a strong odor. The yield of *P. equisetiforme* EO was about equal to the documented yields from *P. aviculare* (0.9%), *P. cognatum* (0.8), *P. lapathifolium* (0.9%), *P. bellardii* (0.8%), *P. persicaria* (0.8%), and *P. arenastrum* (0.9%). However, this yield was larger than the EO produced from *P. arenarium* (0.6%) [30] and *P. minus* (0.30%) [31]. On the contrary, *P. bistorta* subsp. *Carneum*'s flowers have been shown to produce EO with a yield of 0.12% [32], which is higher than the EO produced by *P. equisetiforme* in the current study and by other *Polygonum* plants [30,31].

Totaling 96.48% of the total oil mass, 43 components from *P. equisetiforme*s EO were identified. All the identified components from the GC-MS experiments (Figure 2) were added to Table 1 along with their retention times (Rt), relative abundances, and Kovats indices (KI). Nine categories of compounds were assigned, including the following: oxygen-containing mono- (0.85%), sesqui- (51.98%), and diterpenes (1.29%); non-oxygenated mono- (3.85%), sesqui- (17.08%), and diterpenes (1.08%); non-oxygenated hydrocarbons (14.38%); and carotenoid-derived compounds (1.18%).

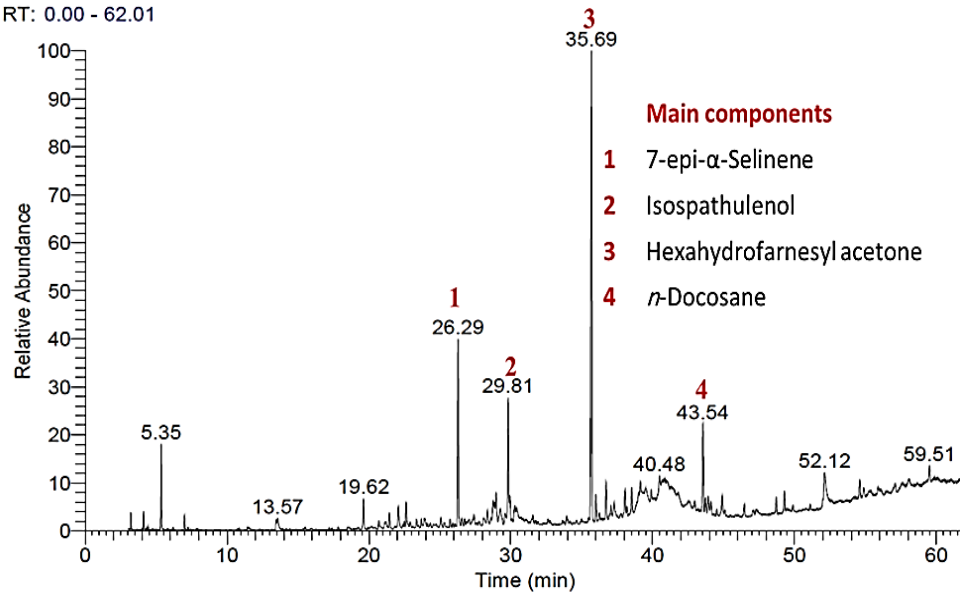

Figure 2. The GC-MS chromatogram of *P. equisetiforme* essential oil. The major component peaks were numbered from 1 to 4.

Table 1. Essential oil chemical composition characterization of *P. equisetiforme*.

Rt	RA (%)	Compound	Class	$KI_{it.}$	$KI_{cal.}$	IM
3.22	0.32 ± 0.01	α-Pinene	MH	939	937	KI and Ms
4.12	0.49 ± 0.02	β-Pinene	MH	979	980	KI and Ms
5.35	2.55 ± 0.07	d-Limonene	MH	1029	1032	KI and Ms
7.00	0.49 ± 0.01	α-terpinolene	MH	1088	1089	KI and Ms
13.46	0.53 ± 0.01	Cumin aldehyde	OM	1241	1243	KI and Ms
13.57	0.56 ± 0.02	β-Longipinene	SH	1400	1399	KI and Ms
19.62	1.48 ± 0.05	α-Cedrene	OS	1411	1410	KI and Ms
21.45	0.74 ± 0.03	*trans*-Caryophyllene	SH	1419	1417	KI and Ms
22.08	1.08 ± 0.04	Phytane	DH	1425	1425	KI and Ms
22.62	1.18 ± 0.05	(*E*)-α-Ionone	Car	1430	1432	KI and Ms
22.92	0.32 ± 0.01	Neryl acetone	OM	1436	1438	KI and Ms
23.35	0.44 ± 0.02	*n*-Pentadecane	H	1500	1498	KI and Ms
23.69	0.38 ± 0.01	(*Z*)-α-Bisabolene	SH	1507	1506	KI and Ms
23.92	0.55 ± 0.02	Modhephen-8-β-ol	SH	1513	1511	KI and Ms
25.72	0.40 ± 0.01	γ-Cadinene	SH	1513	1513	KI and Ms
26.29	14.45 ± 0.12	7-epi-α-Selinene	SH	1522	1524	KI and Ms
26.54	0.48 ± 0.01	*E*-Nerolidol	OS	1563	1561	KI and Ms
27.41	0.56 ± 0.01	Hexahydrofarnesol	OS	1563	1563	KI and Ms
28.09	0.51 ± 0.01	Spathulenol	OS	1578	1576	KI and Ms
28.36	0.72 ± 0.02	Caryophyllene oxide	OS	1583	1584	KI and Ms
28.75	1.16 ± 0.04	Veridiflorol	OS	1590	1588	KI and Ms
28.86	0.96 ± 0.03	Globulol	OS	1590	1592	KI and Ms
28.96	1.54 ± 0.06	*n*-Hexadecane	H	1600	1602	KI and Ms
29.27	1.60 ± 0.07	Humulene epoxide II	OS	1608	1609	KI and Ms
29.81	8.35 ± 0.11	Isospathulenol	OS	1623	1620	KI and Ms
29.93	1.51 ± 0.05	Cubenol	OS	1646	1644	KI and Ms
30.27	1.70 ± 0.06	ar-Turmerone	OS	1669	1671	KI and Ms
32.62	0.33 ± 0.01	Hexadecanal	OH	1819	1821	KI and Ms
35.69	29.45 ± 0.23	Hexahydrofarnesyl acetone	OS	1846	1846	KI and Ms
35.99	1.38 ± 0.12	2-Heptadecanone	OH	1875	1876	KI and Ms
36.25	0.38 ± 0.01	*n*-Nonadecane	OH	1900	1900	KI and Ms
36.72	1.92 ± 0.04	5*E*,9*E*-Farnesyl acetone	OS	1913	1915	KI and Ms
37.28	0.93 ± 0.01	Methyl palmitate	OH	1921	1920	KI and Ms
38.05	1.58 ± 0.05	Carissone	OS	1927	1929	KI and Ms
38.52	1.29 ± 0.03	Phytol	OD	1943	1942	KI and Ms
38.99	0.34 ± 0.01	*n*-Eicosane	H	2000	2002	KI and Ms
39.14	1.36 ± 0.04	9-Octadecenoic acid (*Z*)-, methyl ester	OH	2085	2088	KI and Ms
40.48	1.57 ± 0.04	*n*-Heneicosane	H	2100	2100	KI and Ms
42.58	0.41 ± 0.01	9,12-Octadecadienoic acid (*Z*,*Z*)-, methyl ester	OH	2101	2103	KI and Ms
43.54	6.79 ± 0.09	*n*-Docosane	H	2200	2200	KI and Ms
43.9	1.67 ± 0.05	*n*-Pentacosane	H	2500	2498	KI and Ms
44.89	1.08 ± 0.04	*n*-Hexacosane	H	2600	2603	KI and Ms
54.59	0.95 ± 0.03	*n*-Dotriacontane	H	3200	3200	KI and Ms
	3.85	Monoterpene hydrocarbons (MH)				
	0.85	Oxygen containing monoterpenes (OM)				
	17.08	Sesquiterpene hydrocarbons (SH)				
	51.98	Oxygen containing sesquiterpenes (OS)				
	1.08	Diterpene hydrocarbons (DH)				
	1.29	Oxygen containing diterpenes (OD)				
	14.38	Hydrocarbons (H)				
	4.79	Oxygen containing hydrocarbons (OH)				
	1.18	Carotenoid-derived compounds (Car)				
Total	96.48					

Rt: Retention time, RA (%): relative area abundance, KI: Kovats index (documented (Lit.) and calculated (Calc.), IM: Identification method.

The chemical characterization of *P. equisetiforme*s EO revealed that sesquiterpenes are the essential category of compounds with a relative abundance of 51.98 including non-oxygenated and oxygenated forms. Hexahydrofarnesyl acetone is a major sesquiterpene in the EO of *P. equisetiforme*, with a relative content of 29.45, despite being reported to be minor in the EO of *Polygonum* species as *P. bistorta* subsp. *carneum* [32]. With a relative abundance of 14.45, the 7-epi-selinene was assigned to be the main component in all identified sesquiterpene, particularly the sesquiterpene hydrocarbons. While isospathulenol, with a relative abundance of 8.35, has been identified as the most prominent oxygenated sesquiterpene. According to data from EOs generated from the *Polygonum* species: *P. aviculare, P. persicaria*, *P. lapathifolium*, *P. arenarium*, *P. bellardii*, *P. arenastrum*, and *P. cognatum*, the sesquiterpene abundance in the current investigation was in full accordance with those data.

The recorded data for *P. minus* [31] and *P. bistorta* subsp. *carneum* [32], which include the hydrocarbons as majors, contradicted this finding. The two major sesquiterpenes, 7-epi-selinene and isospathulenol, were not found in any of the *Polygonum* plant's EOs [30–32]. Meanwhile, they were discovered as major constituents of EOs of other plants such as *Ruilopezia bracteosa* [33], *Teucrium yemense* [34], *Piper lepturum* var. *angustifolium* [35], *Piper oradendron* [36], and others.

The current findings include non-oxygenated hydrocarbons as majors and minors of oxygenated forms with a significant abundance of 19.17%. Among all identified hydrocarbons, *n*-docosane (6.79%) and *n*-pentacosane (1.67%) had the highest abundances. In accordance with the present findings, the hydrocarbons were identified as the predominant components in the EOs of a number of *Polygonum* species, including *P. minus* [31], *P. cognatum* [30], and *P. bistorta* subsp. *carneum* [32].

Monoterpenes and compounds-derived carotenoids were all detected as traces in relative quantities of 4.70% and 1.18%, respectively. As traces in the EOs of various *Polygonum* plants, monoterpenes, and carotenoid-derived compounds have also been reported [30–32]. Apart from d-limonene as the main identified monoterpene with a relative abundance of 2.55, the other monoterpenes were identified with minor abundances ranging from 0.32 to 0.53%. Also, only one carotenoid-derived compound, (*E*)-α-ionone (rel. conc. 1.18%), was assigned.

The diterpenes were discovered as a minor category of constituents among terpenoid compounds, with a relative abundance of 2.37%. All of the defined diterpenes were represented by phytol and phytane, with respective percentages of 1.29% and 1.08%. The larger, biosynthesized isoprenoids, such as the diterpenes, are usually non-volatile or hardly volatile, notwithstanding the fact that most isoprenoids, such as isoprenes and mono- and sesqui-terpenes, are extremely volatile chemicals [37,38]. Thus, the enriched EOs with diterpenes are extremely uncommon in the plant kingdom [39]. The minor diterpene constituents in the EO of this plant is fully agreed with this fact and with the scarcity of diterpenes the in EOs of *Polygonum* species [30–32]. Phytol was earlier recognized to be essential in the EO of *P. hydropiper* [30].

3.2. Chemosystematic Significance via Multivariate Analysis

The major compounds of the *P. equisetiforme*s EO (>2.5%) and those of the other *Polygonum* species, including *P. odoratum*, *P. lapathifolium*, *P. arenarium*, *P. aviculare*, *P. arenastrum*, *P. bellardii*, *P. cognatum*, *P. persicaria* [30], *P. minus* [31], and *P. bistorta* subsp. *carneum* [32], were used to construct the chemo-systematic significance of the species. The foundation of this work was the multivariate analysis of the principal components of the various *Polygonum* plants, including principal component analysis (PCA) (Figure 3), hierarchical clustering, and constellation plot (Figure 4). Also, the correlation between the different studies *Polygonum* species and their essential oils compounds are presented in Figure S1.

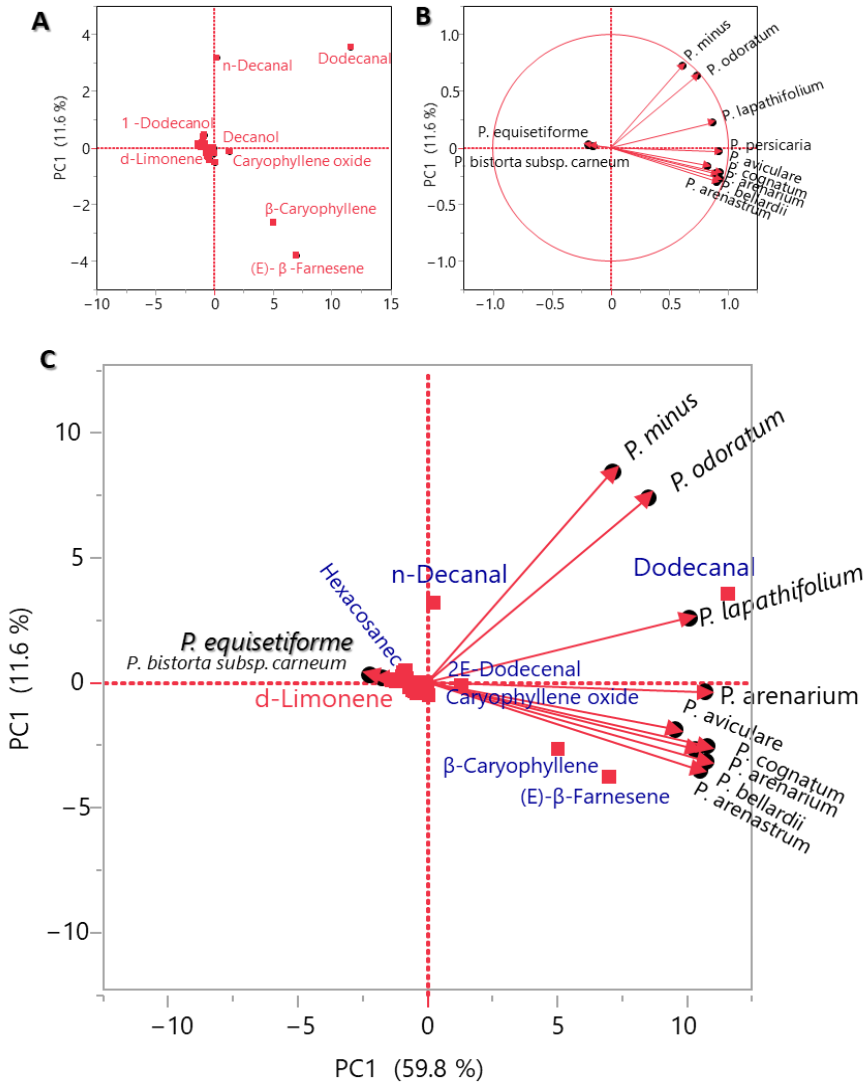

Figure 3. PCA of main constituents of EO of *P. equisetiforme* and the Polygonum species: the PCA space observation (**A**), the correlation circle (**B**), and the biplot diagram (**C**).

The PCA analysis showed that *P. equisetiforme* has a weak association (+11.5%) with the other *Polygonum* species. The findings demonstrated that the *P. equisetiforme*s EO has a distinctive phenomenon of the existence of sesquiterpenes as the primary components with plenty of hexahydrofarnesyl acetone, 7-epi-selinene, and isospathulenol. This outcome was found in the same line of EOs generated from *P. lapathifolium*, *P. arenarium*, *P. aviculare*, *P. arenastrum*, *P. bellardii*, *P. cognatum*, *P. persicaria* [30], and *P. minus* [31]. The main components in some of these *Polygonum* species have been identified as sesquiterpenes, but the main compounds were different than the EO of *P. equisetiforme*.

The main constituents of the EOs of *P. lapathifolium*, *P. arenarium*, *P. aviculare*, *P. arenastrum*, *P. bellardii*, *P. cognatum*, and *P. persicaria* have been found to be β-caryophyllene and (*E*)-β-farnesene [30]. Caryophyllene oxide was also identified as an essential sesquiterpene in the plants *P. lapathifolium*, *P. arenarium*, *P. bellardii*, and *P. persicaria* [30]. On the other hand, the abundance of sabinene, γ-terpinene, geranyl acetate, and α-terpineol in *P. aviculare*, *P. bellardii*, *P. cognatum*, and *P. arenastrum* EOs did not match with the scarcity of monoterpenoids in *P. equisetiforme*s EO in any way [30]. In contrast to the *P. equisetiforme*s EO, which was characterized by the presence of the hydrocarbons as minors, the *Polygonum* species were distinguished by an abundance of the hydrocarbons, such as dodecanal, n-decanal, tetradecanoic acid, and hexadecanoic acid [30]. The ten *Polygonum* were evaluated using

a hierarchical clustering and constellation plot (Figure 4), which provided groupings of plants based on the correlation. Dodecanal, (*E*)-β-farnesene, β-caryophyllene, caryophyllene oxide, and β-bisabolene abundances were observed to significantly correlate with each other in *P. lapathifolium* and *P. bellardii* [30]. *P. arenarium* and *P. minus* displayed a substantial association depending on the presence of oxygenated hydrocarbons like *n*-decanal and decanol [31]. Similar to this, a strong association between *P. arenarium* and *P. persicaria* was established based on the abundance of dodecanal, (*E*)-β-farnesene, β-caryophyllene, and caryophyllene oxide [31]. In conclusion, *P. equisetiforme* has varying correlations with the other *Polygonum* plants, but all our findings are in agreement that the EOs contain high amounts of sesquiterpenes and hydrocarbons.

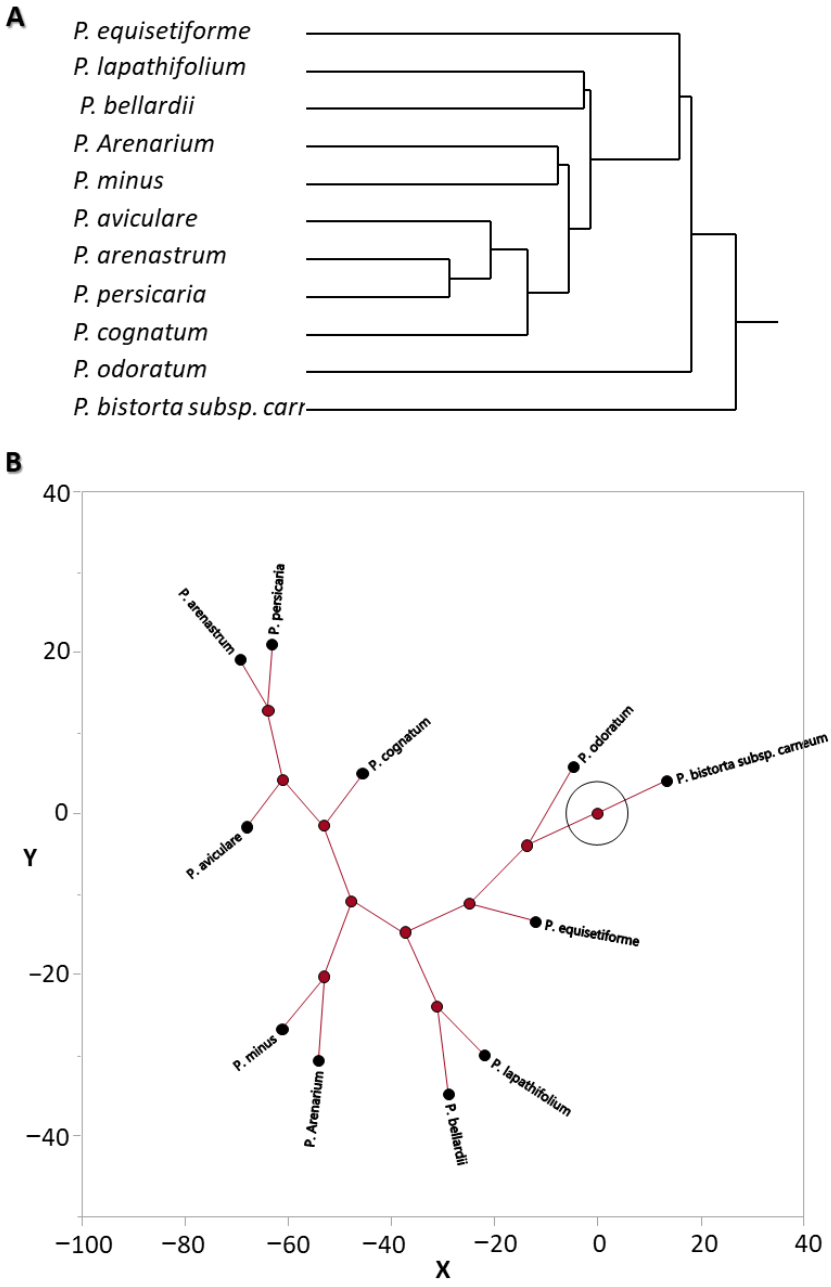

Figure 4. Cluster analysis of the *P. equisetiforme* and another 10 *Polygonum* species based on the abundance of the essential oil compounds: (**A**) hierarchical clustering and (**B**) constellation plot.

3.3. Radical Scavenging Activity

Both the DPPH and ABTS techniques were used to evaluate the EO of *P. equisetiforme* in comparison to vitamin C, a commonly prescribed antioxidant drug (Figure 5). With an increase in EO concentration, it was found that the DPPH radical scavenging increased. The DPPH colors were reduced within the EO concentration at 65.5 and 1000 mg L^{-1} by 20.10% and 67.16%, respectively. On the opposite side, the ABTS colors were lowered by ratios of 38.22% and 73.49%, respectively, using the same oil concentration. However, the concentrations of 12.5 and 125 mg L^{-1} of vitamin C, respectively, showed a reduction in the DPPH and ABTS colors of 42.60% and 84.03% (Figure 5). As a result, this EO behaves like a dose-dependent free radical scavenger.

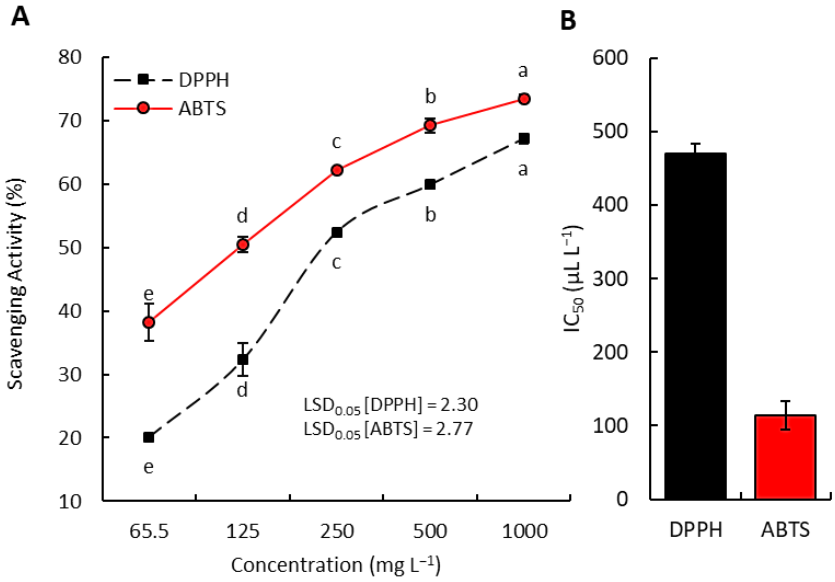

Figure 5. Antioxidant activity of *P. equisetiforme* EO: (**A**) concentration–scavenging activity relation; (**B**) inhibitory concentration of 50% (IC_{50}). Different letters of each line mean significant difference at $p < 0.05$.

Aggregate results revealed that *P. equisetiforme* EO has an efficient DPPH free radical scavenging capacity with an IC_{50} value of 470.01 mg L^{-1}. At an IC_{50} value of 113.74 mg L^{-1}, this oil was found to have a considerable ability to scavenge ABTS radicals. Meanwhile, the findings showed that vitamin C, the reference antioxidant used in this study, exhibits IC_{50} values of 39.06 mg L^{-1} for DPPH and 26.09 mg L^{-1} for ABTS, respectively (Figure 6).

Since each of the richer OH, NH, and SH components contains an active hydrogen atom, the one-step H-atom transfer (HAT) technique offers a potential mechanism for their function as antioxidants [40]. It was also reported that the two assays' mechanisms depend on an equal combination of hydrogen atom transfer (HAT) and electron transfer (ET), while the DPPH reacted with free OH in aromatic acids with an unsubstituted OH group but the ABTS did not distinguish between phenolic OH types [41,42]. This fact is the main strategy of the action mechanism in both DPPH and ABTS techniques. From our findings, the oxygenated constituents represented the main components of *P. equisetiforme* EO, and thus this oil acts as an antioxidant agent. The aliphatic hydroxylated compounds did not react as the action of aromatic acids due to the difficulty of the reaction of the aliphatic OH. So, the increasing of oxygenated components in the EOs lead to an increase in the chance of reaction among the OH groups enabling the radical scavenging ability [43].

Herein, the influence of essential components, such as hexahydrofarnesyl acetone, 7-epi-α-selinene, isospathulenol, and *n*-docosane, may be responsible for the free radical scavenging features of *P. equisetiforme* EO. These main compounds, along with the minor components, might function individually or together in a synergistic manner [44,45]. Hexahydrofarnesyl acetone has been identified as a main sesquiterpene found in multiple

plants, including *Launaea* species [46], *Kickxia aegyptiaca* [47], *Heliotropium curassavicum* [48], and *Bassia muricata* [49], which are all sources of powerful antioxidant essential oils. The EOs derived from *Eugenia uniflora* and *Cymbopogon schoenanthus* were reported to have a significant antioxidant effect due to the presence of 7-epi-α-selinene as one of its main components [50,51]. Spathulenol and its isomer are very significant antioxidant agents in plants' EOs [52]. To enable further investigation into the action of these main compounds as antioxidant agents, an in-silico study using MOE software was performed into hematopoietic cell kinase (Hck) and human Peroxiredoxin 5.

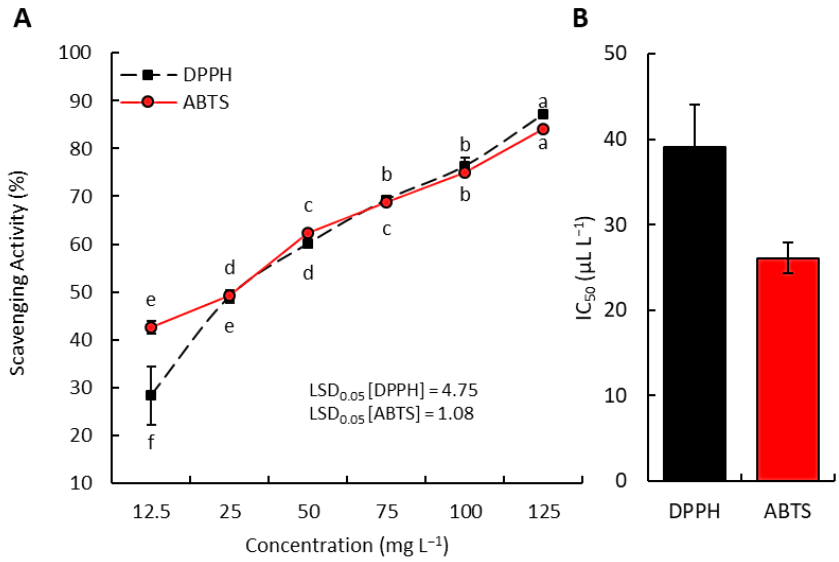

Figure 6. Antioxidant activity of vitamin C (standard antioxidant): (**A**) concentration–scavenging activity relation; (**B**) inhibitory concentration of 50% (IC_{50}). Different letters of each line mean significant difference at $p < 0.05$.

3.4. Molecular Docking Studies

The main metabolites of the EO have been docked into hematopoietic cell kinase (Hck) and human Peroxiredoxin 5 using MOE software. The results revealed remarkable affinities of the oxygenated sesquiterpenes toward both proteins as presented in Table 2. Compared to the reference compound, ascorbic acid, all studied compounds showed good binding affinity to Hck with a range of ΔG at −4.93 to −7.08 kcal/mol. Farnesyl acetone and hexahydrofarnesyl acetone revealed the best binding affinity with ΔG = −7.08 and −6.83 kcal/mol in comparison to ascorbic acid with ΔG = −5.12 kcal/mol.

Table 2. Results of docking studies of the main identified compounds of the essential oil to the active sites of hematopoietic cell kinase (Hck) and human Peroxiredoxin 5. [a] binding energy.

Compound	ΔG [a] (kcal/mol)	
	Tyrosine Kinase Hck	Human Peroxiredoxin 5
D-Limonene	−5.17	−4.46
7-*epi*-α-Selinene	−4.93	−4.86
Isospathulenol	−5.41	−4.70
Cubenol	−5.41	−4.84
ar-Turmerone	−6.14	−4.93
Hexahydrofarnesyl acetone	−6.83	−5.34
5*E*,9*E*-Farnesyl acetone	−7.08	−5.47
Carissone	−5.35	−4.77
n-Docosane	−6.79	−5.25
Vitamin C (reference)	−5.12	−4.32

The acetonic carbonyl of both compounds formed hydrogen bonds with the crucial amino acids Lys 295 and Met 341. Additionally, hydrophobic interactions were formed between an aliphatic chain of both compounds and the amino acids residues Leu 273 and Val 281 (Figure 7).

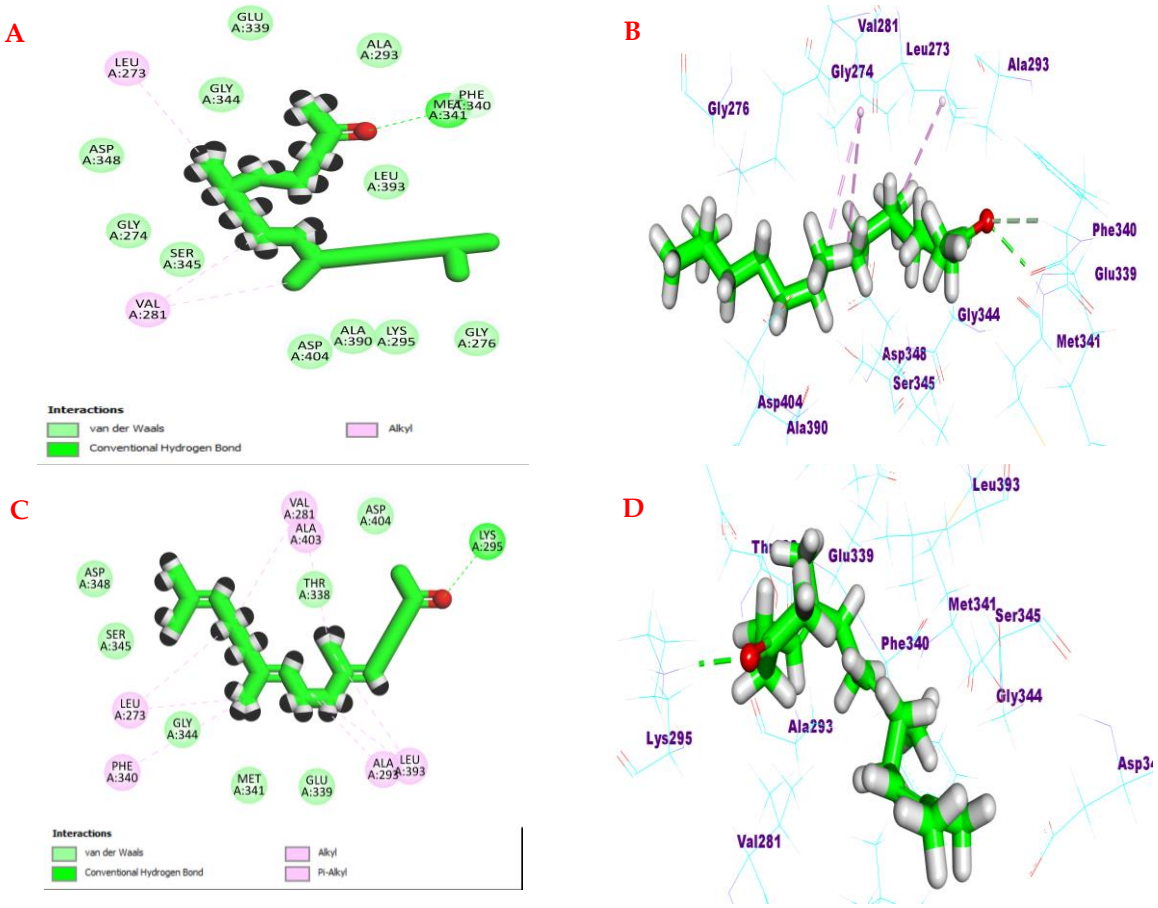

Figure 7. (**A**,**C**) Two-dimensional binding modes and (**B**,**D**) three-dimensional binding modes of compounds hexahydrofarnesyl acetone and farnesyl acetone in the active site of hematopoietic cell kinase (Hck) (PDB ID: 2HCK).

Similarly, the investigated compounds exhibited remarkable binding affinity to human Peroxiredoxin 5 with ΔG range −4.46 to −5.47 kcal/mol compared to the ascorbic acid ΔG = −4.32 kcal/mol. In the same way, oxygenated sesquiterpenes, including ar-turmerone, hexahydrofarnesyl acetone, and farnesyl acetone, showed the highest affinity with ΔG = −5.47, 5.34, and −4.93 kcal/mol, respectively. With the crucial amino acid Gly 46, the carbonyl group of three compounds formed hydrogen bonds. Besides, other hydrophilic interactions were formed between the amino acids and three compounds (Figure 8).

This study pointed out that the antioxidant effect of EO is not restricted only to the phenolic compounds but also to the non-phenolic terpenoids. Baschieri and his coworkers reported that the non-phenolic terpenoids have termination-enhancing antioxidant activity. This antioxidant performance requires higher concentrations than that of phenols. Also, it is much less predictable due to non-concentration linearity [53].

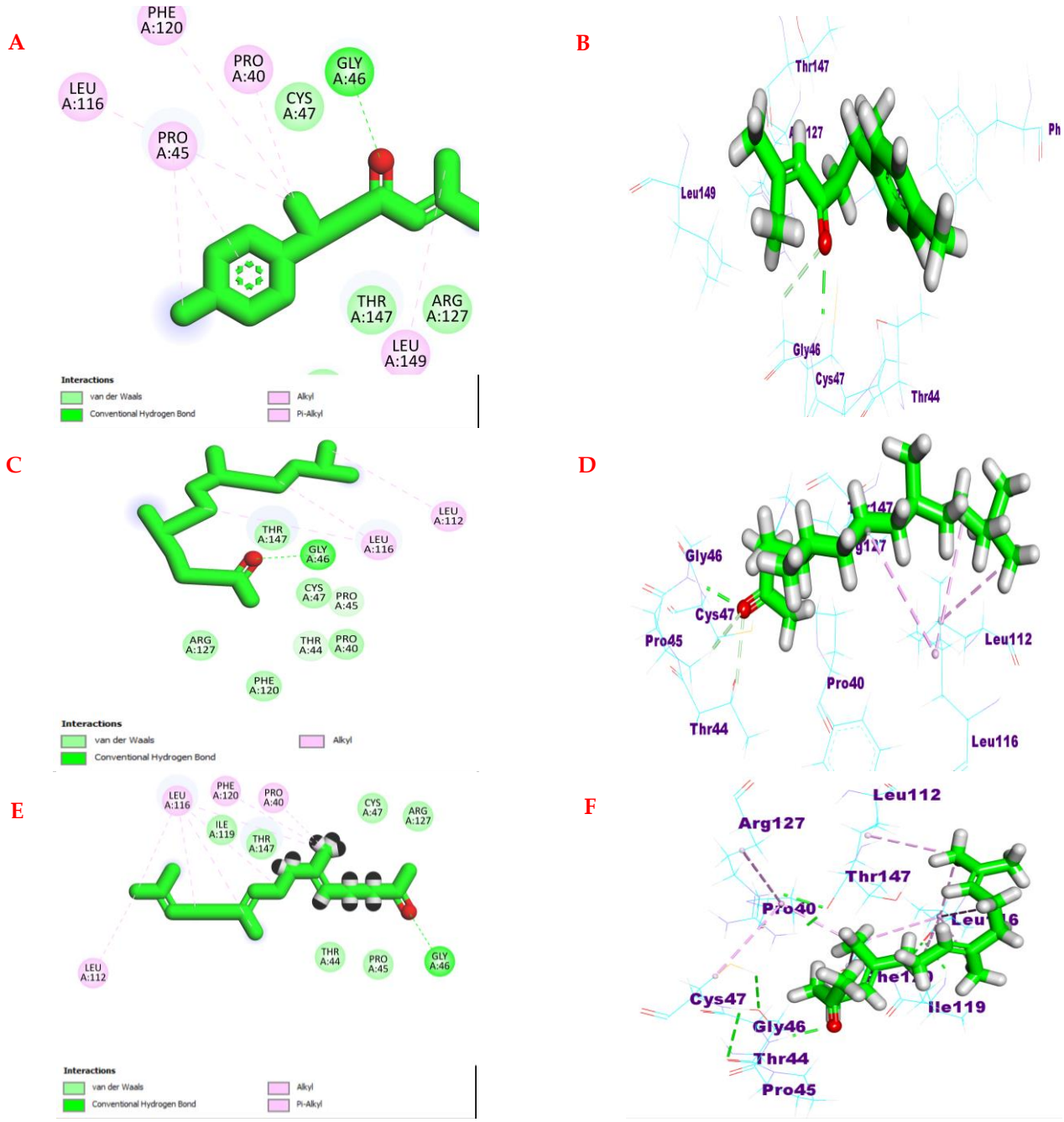

Figure 8. (**A**,**C**,**E**) Two-dimensional binding modes and (**B**,**D**,**F**) three-dimensional binding modes of compounds ar-turmerone, hexahydrofarnesyl acetone, and farnesyl acetone in the active site ofhuman Peroxiredoxin 5 (PDB ID: 1HD2).

4. Conclusions

The EO of *P. equisetiforme* was extracted via the hydrodistillation technique. The current GC-MS profiling of *P. equisetiforme* EO afforded the identification of terpenes as main constituents. Hexahydrofarnesyl acetone, 7-epi-selinene, isospathulenol, and *n*-docosane were assigned as the major compounds. The chemometric systematic significance of *P. equisetiforme* with different *polygonum* plants depending upon the PCA and AHC revealed that this plant has a characteristic chemical fingerprint among all studied plants. The free radical scavenging assaying results revealed that *P. equisetiforme* EO has efficient DPPH and ABTS radical scavenging abilities compared to vitamin C as a standard antioxidant. The plausible chemo-biological study revealed the significant antioxidant action contribution

of the main EO compounds, either singly or in combination. This result was deduced via the MOE study that exhibited the significance of the oxygenated sesquiterpenes especially ar-turmerone, hexahydrofarnesyl acetone, and *5E*,*9E*-farnesyl acetone. The here-presented data highlight the need for additional research on the biological functions of this EO and its constituent parts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry5040151/s1, Figure S1: Correlation matrix between the different studies *Polygonum* species and their essential oils compounds.

Author Contributions: Conceptualization, A.M.A.-E. and A.I.E.; formal analysis, A.M.A.-E., R.F.A., A.F.E., A.E.-N.G.E.G., S.A.E.-N., A.I.E. and Y.A.E.-A.; investigation, A.M.A.-E., R.F.A., A.F.E., A.E.-N.G.E.G., S.A.E.-N., A.I.E., T.C.S. and Y.A.E.-A.; writing—original draft preparation, A.M.A.-E., R.F.A., A.F.E. and A.I.E.; writing—review and editing, A.M.A.-E., R.F.A., A.F.E., A.E.-N.G.E.G., S.A.E.-N., A.I.E., T.C.S. and Y.A.E.-A.; visualization, A.M.A.-E. and A.I.E. All authors have read and agreed to the published version of the manuscript.

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