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Chemical profile, biological activities, and molecular docking of Algerian *Juniperus phoenicea* berries

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Abstract

The chemical composition, antioxidant activities, and α-amylase enzyme inhibitory activity of Algerian *Juniperus phoenicea* L berries were quantitatively and qualitatively determined in this study. Essential oil (EO) and non-polar crude extracts from cyclohexane and ethyl acetate were prepared, and the chemical profile was determined using GC-MS technique. The predominant compound in the EO was α-pinene (76.03%), while communic acid (23.66% and 22.38%) was the main compound in both non-polar crude extracts. The antioxidant potential of the samples was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)-diammonium salt (ABTS), and phenanthroline. All samples showed weak antioxidant capacity. The antidiabetic effect was assessed *in vitro* using the α-amylase assay; a strong inhibitory effect against the α-amylase enzyme was detected for both cyclohexane and ethyl acetate extracts with IC_{50} (IC_{50} = 186.91 ± 5.74 mg/mL and IC_{50} = 351.48 ± 0.17 mg/mL, respectively). Finally, an *in silico* study was performed for both α-amylase and α-glucosidase proteins to enhance our outcomes.

Keywords: α-amylase assay; anti-oxidant assays; essential oil; *Juniperus phoenicea* L berries; molecular docking; non-polar extracts

Introduction

The medicinal properties of plants have been widely known and used since ancient times due to their beneficial and curative effects. A medicinal plant's distinct pharmacological qualities stem from the combined effects of its many natural elements. The whole expression of a medicinal plant's bioactivity is found in

its plant complex, which is a biochemical entity (Bouras *et al*., 2024). In fact, its synergistic and additive effects, increased pharmacological efficacy, or even decreased toxicity, are often not related to a single active component (Severina *et al.*, 2013). Essential oils (EOs) are volatile compounds with a low molecular weight and biological activities synthesized in different plant organs, especially flowers, buds, leaves, branches, stems, seeds, fruits, woods, and roots (Jain *et al.*, 2022). Furthermore, an abundance of active chemicals, including alkaloids, tannins, steroids, glycosides, resins, phenols, volatile oils, and flavonoids, are present in EOs (Amirifar *et al*., 2022; Asgari *et al*., 2017).

Nowadays, plant extracts and EOs are sources of advantageous chemical compounds with potential uses in the food, cosmetics, pharmaceutical, and agricultural industries. They are becoming popular natural alternatives to synthetic antioxidants to address consumer concerns about the adverse effects of synthetic antioxidants that have toxic effects on consumers and can lead to various cancers. Therefore, new and affordable sources of natural antioxidants are becoming more widely available in order to preserve and improve customers' health and ensure food safety (Nastaran *et al*., 2021; Zahnit *et al*., 2022). Due to the significant amounts of volatile, aromatic, and bioactive chemicals they contain, EOs and extracts are highly valued in various sectors (Messaoudi *et al*., 2022; Samadi *et al*., 2021). Additionally, these effective compounds have inherent antioxidant, enzymatic, and antibacterial characteristics that are crucial for daily life (Bolouri *et al*., 2022).

Around the world, *Juniperus* species are frequently used in traditional medicine for a variety of purposes. Among their many uses, they are used as insect repellent, hypoglycemic, carminative, diuretic, antibacterial, antitussive, antifertility, and stomachic. However, they are also favored as treatments for urticaria, rheumatoid arthritis, TB, leukorrhea, fever, diarrhea, jaundice, and urinary tract infections. Berries of the *J. phoenicea* plant are the source of *juniper* oil, which is included in various pharmacopeias, including the 8th edition of the European Pharmacopoeia (Ph. Eur. 8). For centuries, *J. phoenicea* berries and their EOs have been used for both medicinal and cosmetic purposes, due to their complex combination of chemical compounds, mostly aromatics and terpene hydrocarbons, in addition to terpenoids, polyphenolic compounds, alcohols, myrcene, thujone, glycoside acetate-ethers, citronellol, curcumin, geraniol, terpineol, and ketones. According to Amalich *et al.* (2015), the primary chemical found in the EO of Moroccan *J. phoenicea* was α-pinene (78.8%), germacrene D (5.42%), 4 trans-data-4(11),7-diene (2.98%), E-caryophyllene (2.77%), α-himachalene (0.9%), and δ- cadinene (0.71%). Also, in the study by Mehira *et al*. (2021), which compared EO extractions from leaves and a combination of leaves/berries, a significant increase in the amount of chemical composition was found for the combination of leaves/berries. In the same context, El-Sawi *et al.* (2007) reported, in their study, the chemical composition of Egyptian *J. phoenicea* berries oil, where they found the major components were α-pinene (39.30%) followed by sabinene (24.29%), trans-pinocarveol (4.27%), β-phellandrene (4.13%), α-terpinyl acetate (3.36%), p-mentha-1,5-diene-8-ol (2.88%), and β-pinene (2.45%). Additionally, Medini *et al*. (2011) examined the chemical composition and antioxidant activity of the EO of Tunisian *J. phoenicea L* ripe and unripe berries. They indicated that the major components of the oils were α-pinene (58.61%–77.39%), camphene (0.67%–9.31%), δ-3-carene (0%–10.01%), and trans-verbenol (0%–5.24%). Also, Ennajar *et al*. (2009) analyzed the chemical composition of *J. phoenicea* plants collected from the southeastern region of Tunisia. They found that the main components were α-pinene (80.7%), δ-3-carene (4.5%), γ-cadinene (5.1%), β-caryophyllene (2.9%), β-myrcene (1.8%), and germacrene B (1.5%). Another study conducted by Bouyahyaoui *et al*. (2016) investigated the chemical composition of Algerian *J. phoenicea* EO from berries. They found that the main constituents were α-pinene (56.6%), α-terpineol, myrcene (2%), verbenone (1.9%), germacrene D (1.5%), β-caryophyllene (1.2%), camphor (1.9%), p-mentha-1,5-dien-8-ol (1.6%), terpinen-4-ol (1.1%), trans-pinocarveol (1.1%), α-phellandrene (1%), and β-pinene (1%). Harhour *et al*. (2018) analyzed the composition of the EOs of wild *J. phoenicea* from northern Algeria, including berries and branches. They found that the major components were α-p (40.3%), δ-3-carene (20.1%), α-cedrol (7%), α-terpinolene (4.5%), β-phellandrene (4.1%), β-myrcene (2.8%), α-terpinylacetate (2.7%), β-caryophyllene (1.9%), α-fenchene (1.9%), α-humulene (1.6%), β-pinene (1.5%), and elemol (1.2%).

The novelty of the present study is the enhancement of the chemical data from two non-polar crude extracts (cyclohexane and ethyl acetate). This information was obtained for the first time using GC-MS techniques to the best of our knowledge. The main purpose of this study was to characterize the chemical composition of the EO and non-polar extracts (cyclohexane and ethyl acetate) isolated from *J. phoenicea* berries. These berries were harvested in the Aflou region of Laghouat (Algerian Sahara). The study aims to assess their biological activities, including anti-diabetic effects (α-amylase activity) and antioxidative properties (DPPH, ABTS, and phenanthroline activities). Additionally, outcomes were further analyzed with molecular docking and absorption, distribution, metabolism, elimination, and toxicity (ADMET) studies. These analyses aimed to investigate the binding affinity of all compounds identified through GC-MS analysis to the active sites of α-amylase and α-glucosidase. Furthermore,

the pharmacokinetic properties of each extract were determined through ADMET experiments. Ultimately, the data synthesized through these experiments provided insight into the best-docked molecules.

Materials and Methods

Plant material

Ripe berries of *Juniperus phoenicea* were collected in March 2023 from the region of Aflou (Djebel Amour), located at the center of the Saharan Atlas and at the edge of the Sahara Desert (34°12′ N; 2°10′ E, 1300–1470 m) in the Laghouat province of southern Algeria. The plant's identity was verified by the Department of Biological Sciences, Faculty of Science at Laghouat University in Algeria. Voucher specimens were deposited at the Research Unit of Medicinal Plant at the University of Laghouat, Algeria with the identification numbers URPM. Jp.03.23.

The *J. phoenicea* ripe berries were separated and placed on paper. The samples were dried in two steps: shade-drying and oven-drying.In the case of shade-drying, initially, 700 g of fresh sample was spread over 1 m^2 of area and dried in a ventilated place at a temperature of 25 ± 2°C for 3 weeks. The same quantity was then transferred to an oven and dried for 4 days at a temperature of 50°C with 40% ventilation. The plant materials were ground using a basic grinder (IKA-10) with parameters of 3000rpm and a 1 mm sieve to get a homogenous powder.

To calculate the moisture content of the sample, 5 mg of ground fruit was placed in a porcelain mortar in the oven at a temperature of 105°C without ventilation for 24 h. After that, the moisture content (TH%) was determined to be 5.3%.

Preparation of plant extracts

Essential oil extraction

Juniperus phoenicea ripe berries (300 g) were ground into small pieces and subjectedto hydro-distillation for 3 h in a Clevenger-type apparatus, using 1 L of deionized water. The transparent oil was collected, dried over anhydrous Na_2SO_4, and stored at 4°C in dark sealed vials for further analysis.

Non-polar crude extracts

Forty grams (40 g) of dried berry powder was divided into two equal parts (20 g each) and separately extracted by maceration with 100 mL of solvents (cyclohexane and ethyl-acetate) in a 1:5 ratio. This extraction process was repeated three times with fresh solvent each time. The mixture was then filtered through Whatman filter paper no.1. The filtrate was evaporated at 40°C using a rotary evaporator and stored in a refrigerator at 4°C for further analysis.

Reagents and chemicals used

At the National Center for Biotechnology Research, we used a 96-well microplate reader (PerkinElmer Multimode Plate Reader EnSpire) to measure the activities of various substances. These substances included ascorbic acid, quercetin, α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Folin–Ciocalteu reagent (FCR), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)-diammonium salt (ABTS), phenanthroline, pancreatic α-amylase enzyme (1U), acarbose, starch, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, and hydrochloric acid, all obtained from Sigma-Aldrich. Additionally, we used sodium carbonate, aluminum chloride ($AlCl_3$), iron (III) chloride ($FeCl_3$), sodium bicarbonate ($NaHCO_3$), potassium iodide (KI), potassium persulfate ($K_2S_2O_8$), and potassium acetate (CH_3CO_2K), obtained from Biochem Chemopharma. All other chemicals and solvents used were of analytical grade.

Gas chromatography-mass spectrometry (GC-MS)

The composition of *J. phoenicea* berries' EO and crude extracts was determined using a gas chromatography-mass spectrometer system (GC-MS; GC-2010 Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer detector (QP-2020 A, Shimadzu). The GC–MS system utilized an Rxi®-5ms capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane) with dimensions of 30 m × 0.25 mm and a 0.25 μm film thickness. This column is similar in phase to HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB-1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms and is considered equivalent to USP G1, G2, and G38 phases.

Analyses were conducted using helium (99.995% purity) as a carrier gas at a column flow rate of 1 mL/min with 1 μL of the sample injected in split mode at a ratio of 1:50. The oven program was as follows: injector and interface temperatures were maintained at 250°C and 310°C, respectively. The column temperature was initially set at 50°C fixed for 2 min, then increased to 310°C at a rate of 3°C/min, and then maintained at 310°C for 2 min.

The mass spectrometer conditions included an ionization voltage of 70 eV, ion source temperature of 200°C, and electron ionization mass spectra acquired over the mass range of 45–600 m/z. Component identification was determined by matching mass spectra with Wiley

and NIST library data from 2017, standards of the main components, comparing Kovats Retention Indices (KRI) with reference libraries, and relevant literature (Adams, 2001; Barrero *et al*., 2005). Component concentrations were determined by semi-quantification through peak area integration from GC peaks and applying the correction factors of homologs series of C_9-C_{10} n-alkanes and available authentic standards. For both EO and crude extract, a similar method was used, with only difference being in the sample volume and split mode (0.5 µL for the crude extract and a split ratio of 1:10).

Colorimetric total phenolic and flavonoid content

Colorimetric total phenolic (CTP)

The Folin–Ciocalteu method, slightly modified by Ostadi *et al*. (2020), was used to assess the total phenolic content, with results expressed in µg of gallic acid per mg of extract.

Colorimetric total flavonoid (CTF)

Following the study by Singleton *et al.* (1999), total flavonoids were calculated using the aluminum chloride method, with results presented in µg of quercetin per mg of extract.

Colorimetric antioxidant capacity

Antioxidants exists in various classes and forms in plants, including flavonoids, coumarins, benzoic acid derivatives, phenolic compounds, and carotenoids. Multiple spectrophotometric methods are used to measure the total antioxidant capacity and content, providing a comprehensive profile of the antioxidant levels and capacity of the substances being studied. This study evaluated the antioxidant potential of EOs and non-polar extracts using six different methods, such as $DPPH^{\bullet}$, phenanthroline, and $ABTS^{\bullet+}$, compared to five standards: BHA, BHT, ascorbic acid, quercetin, and α-tocopherol.

Colorimetric DPPH assay

The DPPH assay was determined using the methodology outlined by Moreno *et al.* (2000). The results were compared to positive standards including BHA, ascorbic acid, quercetin, α-tocopherol, and BHT, with absorbance readings taken at 517 nm. The concentration at which the DPPH radical could be 50% extinguished (IC_{50}) was determined by plotting percentage inhibitions of extinction, as shown in Equation (1). The results indicated that the tested sample exhibited a greater capacity for scavenging free radicals, as evidenced by the decrease in absorbance of the solution:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

where, $A_{control}$ is the absorbance of blank and the A_{sample} is that of extract or standard sample.

Colorimetric phenanthroline assay

Oyaizu (1986) utilized the phenanthroline technique to determine the activity, with the findings reported as $A_{0.50}$ (µg/mL). In order to compare the results obtained, positive standards such as BHA, ascorbic acid, quercetin, α-tocopherol, and BHT were used (Oyaizu, 1986).

Colorimetric ABTS assay

The technique used to assess the $ABTS^{\bullet+}$ activity, and the findings were expressed as IC_{50}, based on the work of Özyürek *et al.* (2012). BHA, ascorbic acid, quercetin, α-tocopherol, and BHT were utilized as positive standards for comparing the acquired findings.

Anti-diabetic assay

Anti-α-amylase assay

This method was performed using the iodine/potassium iodide (IKI) method described by Zengin *et al.* (2014), with a few minor adjustments. A 96-well microplate was incubated for 10 min at 37°C with 50 µL of α-amylase solution (1U) prepared in phosphate buffer [PBS (pH 6.9 supplemented with 6 mM sodium chloride)] combined with 25 µL of each extract dissolved in MeOH, or acarbose as a positive control, at varying concentrations (6.25–400 µg/mL). Subsequently, 50 µL of 0.1% starch solution were added to initiate the reaction. The reaction mixture was then incubated at 37°C for 10 min. The reaction was stopped by adding HCl (25 µL, 1 M) and 100 µL of IKI solution. Similarly, a blank was prepared by adding the sample solution to all reagents without the enzyme α-amylase solution, and acarbose was used as a positive control. Absorbances were measured at 630 nm, with the absorbance of the blank subtracted from that of the samples (Zengin *et al.*, 2014). The α-amylase inhibitory effect was calculated according to Equation (2):

$$\%\ inhibition = 1 - \frac{(A_c - A_e) - (A_s - A_b)}{A_c - A_e} \qquad (2)$$

where,

A_c: Absorbance of the mixture of starch + IKI + HCl + MeOH + phosphate buffer solutions

A_e: Absorbance of the mixture of the enzyme + starch + IKI + HCl+ MeOH solutions

A_s: Absorbance of the mixture of the enzyme + sample + starch + IKI + HCl solutions

A_b: Absorbance of the mixture of the sample + IKI + phosphate buffer solutions.

Molecular Docking

Selection and preparation of protein targets

The crystallographic 3D structures of the studied enzymes: human pancreatic alpha-amylase in complex

with montbretin A (PDB ID: 4W93) and sugar beet alpha-glucosidase with acarbose (PDB ID: 3W37) were retrieved from the Protein Data Bank website (http://www.rcsb.org) in ".PDB" format. The native ligands and water molecules were removed, and polar hydrogen atoms and Kollman charges were added using MGL-AutoDockTools (ADT, v1.5.7) (Morris *et al.*, 2009). This procedure was applied to both proteins and then saved as the dockable protein databank extension ".pdbqt" for docking simulations.

Retrieval and preparation of ligands

The 3D structures of bioactive compounds, detected by GC-MS analysis, were retrieved from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in ".SDF" format. The collected ligands were then converted to the ".pdbqt" format using AutoDock Tools for molecular docking simulation.

Molecular docking execution

Autodock Vina was used for assessing binding affinity. The grid box centers, X, Y, and Z dimensions, used for the studied targets were (−9.632488, 4.340907, −23.107256) (20x20x20) and (0.108773, −1.916977, −23.053182) (20x20x20) for α-amylase (4W93) and α-glucosidase (3W37), respectively. The best binding pose with the lowest binding energy (kcal/mol) was chosen, and the interaction bonds were visualized in 3D and 2D images using Discovery Studio software. These parameters were applied to all studied compounds, and the top three interacted ones for each extract were graphically presented. The docking results were validated by redocking the native inhibitor into the enzyme's active region (Trott and Olson, 2010).

ADMET properties

The study of drug absorption, distribution, metabolism, excretion, and toxicity (ADMET) is known as pharmacokinetics. Eliminating poor drug candidates is a crucial concept that not only determines the availability of a drug but also helps to prevent issues during *in vivo* research (Cheng *et al.*, 2012; Yang *et al.*, 2019). In this study, ADMET characteristics were determined using ADMETSAR.

Results and Discussion

Chemical composition

Essential oil

Hydrodistillation was used to extract the EO of *J. phoenicea* berries, allowing the extraction of 3.33% of the total mass of crude dry plant material., The yellow pale EO of *J. phoenicea* was compared to yields found in the literature, and Table 1 summarizes the different yields

Table 1. Yields and major chemical composition of different *Juniperus phoenicea* berries.

Region	Yield %	Major components	References
Algeria (Aflou)	3.33	α-Pinene (76.03%), Caryophyllene (3.10%) β-Pinene (2.7%), Myrcene (1.73%), Germacrene D (1.7%)	This study
Algeria (Naama)	2	α-Pinene (56.6%), α-Terpineol (3.3%) Camphor (1.9%), Verbanone (1.9%), p-Mentha-1,5-dien-8-ol (1.6%).	Bouyahyaoui *et al.* (2016)
Algeria (Mostaganem)	0.14	α-Pinene (43.7%), p-Cymene (5.8%) β-Phellandrene (4.6%), α-Terpineol (4.3%), Germacrene D (1.7%).	Abdelli *et al.* (2018)
Algeria (Bouira)	2.7	α-Pinene (80.80%), Caryophyllene oxide (3.9%), 3-Carene (1.90%) , β-Pinene (1.8%), α-Humulene oxide (1.6%)	Menaceur *et al.* (2013)
Algeria (Ain Defla)	1.1	α-Pinene (40.30%), 3-Carene (20.10%), α-Terpinolene (4.5%) α-Cedrol (7%), β-Phellandrene (4.1%)	Harhour *et al.* (2018)
Italy (Sardinia)	2.54	α-Pinene (87.54%), 3-Carene (1.23%) β-Phellandrene (2.81%), Myrcene (1.61%), Germacrene D (1.17%).	Angioni *et al.* (2003)
Tunisia (Matmata)	Shade drying (2.82%) Sun drying (3.40%)	May (Shade drying): α-Pinene (26.9%), γ -Cadinene (10.2%), Caryophyllene oxide (6.3%), β-Selinene (6.9%), Germacrene B(7.3%). May (Sun drying): α-Pinene (86.4%), γ -Cadinene (4.6%), β-Myrcene (2.2%), γ-Terpinene (1.6%), Germacrene B (1.1%)	Ennajar *et al.* (2010)
Tunisia (1-Rimel, 2-Makthar, 3-J. Mansour)	2–7	1− α-Pinene (63.40%), Caryophyllene oxide (3.5%), 3-Carene (10%), Trans-verbenol (2.40%), β-Pinene (2%) 2- α-Pinene (69.50%), β-Pinene (2.1%), Camphene (9.3%), Caryophyllene (1.8%), Myrcene (2.1%) 3- α-Pinene (77.3%), β-Pinene (2.5%), β-3-Carene (3.7%), β-phellandrene (2.5%), Trans-verbenol (1.60%)	Medini *et al.* (2011)
Morocco (Tounfite)	2.01	α-Pinene (78.11%), β-Pinene (0.95%), β-Trans-Dauca-4(11),7-diene (2.96%), Caryophyllene (2.77%), Germacrene D (5.42%)	Amalich *et al.* (2015)

found. The chemical composition of the EO was analyzed by GC-MS, with a total of 50 compounds identified (Table 2) totalling 98%. Oxygenated monoterpenes were the most abundant components, despite the main components being derived from the monoterpenes hydrocarbons α-pinene (76.03%), β-pinene (2.75%), myrcene (1.73%), 3-carene (1.15%), and D-limonene (1.32%). Other compounds from sesquiterpene hydrocarbons such as caryophyllene (3.10%), α-humulene (1.25%), and germacrene D (1.7%) were also present. Most of the compounds identified in the EO of *J. phoenicea* berries in this study have been previously described for the same subspecies, although in different amounts.

The berries used in this study were in the same stage of maturation as those reported by the previous authors. α-Pinene predominated in all examined EOs, with levels ranging from 27% to 87.5%, depending on the local, harvesting season, and amount of plant material. As mentioned in the literature, the *phoenicea* group of *juniper* significantly supported these findings. These results are in agreement with those reported by research communities in this field, especially those focusing on this genre in Algeria. The majority of compounds remain the same, with variationsmainly in percentage. Slight differences were reported for some compounds like Dauca-4(11),7-diene that were reported in a study conducted by Amalich *et al.* (2015).

Non-polar extracts

In the present study, and for the first time to our knowledge, we provide a report on the phytochemical composition of cyclohexane and ethyl acetate extracts from *Phoenician juniper*. According to a study by Byrne *et al.* (2016) which categorized green solvents for extractions, separations, formulations, and reaction chemistry, the two solvents selected for this study were the most appropriate based on environmental, health, and safety (EHS) criteria as outlined by the three big pharmaceutical companies Pfizer, GlaxoSmithKline, and Sanofi (Byrne *et al.*, 2016).

Classical maceration was used for 3 days to prepare the crude extract of non-polar solvents (cyclohexane and ethyl acetate) from *Phoenician juniper* berries. This process allowed the extraction of 7.95% and 8.7,% respectively, of the total mass of the crude dry plant material. The variation in yield was attributed to the polarities of the different compounds in the berries, a variation that has been noted in the literature, as seen in the study by Ennajar *et al.* (2009). The total flavonoid content was reported as micrograms of quercetin equivalents per milligram of extract (μg QE/mg) and the total phenolic content was reported as micrograms of gallic acid equivalents per milligram of extract (μg GAE/mg). The results are summarized in Table 2.

The chemical composition of cyclohexane and ethyl acetate extracts was analyzed using the same GC-MS. A total of 89 and 82 compounds were identified (Table 3), with a total percentage of identification of 67% and 68%, respectively. Despite the EO, the most abundant components for the non-polar extract are the oxygenated diterpenes, with amounts of 21.34% and 21.95% respectively. The analysis reveals the appearance of new compounds such as sterol (gamma.-Sitosterol), lignan (Anthricin), ether (Methyl tetratriacontyl ether)

Table 2. Antioxidant activities with phenolic and flavonoid content of nonpolar extracts and essential oil of *Juniperus phoenicea.*

Extracts	DPPH. Assay IC_{50} (μg/L)	Phenoltroline	ABTS.+Assay	Total Phenolics	Total Flavonoids
Essentiel Oil	In	In	In	–	–
Cyclohexane	>800	>800	>800	19.39 ± 0.61	10.96 ± 1.38
Ethyl acetate	>800	>800	378.28 ± 5.18	20.86 ± 2.44	12.93 ± 0.83
BHA	9.11 ± 0.89^{c}	1.49 ± 0.08^{ab}	2.98 ± 0.11^{ab}	NT	NT
BHT	1.60 ± 0.36^{a}	2.20 ± 0.04^{b}	1.31 ± 0.06^{a}	NT	NT
α-Tocopherol	19.99 ± 0.74^{d}	5.78 ± 0.30^{c}	10.54 ± 0.07^{b}	NT	NT
Quercetine	3.40 ± 0.30^{b}	0.65 ± 0.04^{a}	2.50 ± 0.06^{a}	NT	NT
Ascorbic acid	2.69 ± 0.22^{b}	8.30 ± 0.76^{d}	4.04 ± 0.02^{ab}	NT	NT

The concentration at 50% inhibition and the concentration at 0.50 absorbance, respectively, are referred to IC_{50} and $A_{0.50}$ values. By using a linear regression analysis, the IC_{50} and $A_{0.50}$ values were determined and expressed as mean ± SD (n = 3). The values in the same columns that have different superscripts (a, b, c, d, e, f, g, or h) differ significantly ($p < 0.05$).
BHA: utylated hydroxyanisole, BHT: butylated hydroxytoluene, b: reference compounds, BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, Nd: not determined, NT: nottested,
*total phenolics (μg gallic acid/mg extract), **total flavonoids (μg quercetin/mg extract)
Statistical study was made in term of n = 3.

Table 3. Chemical composition of *Juniperus phoenicea* berries essential oils and nonpolar extracts (cyclohexane and ethylacetate); molecular docking score for both enzymes (α-amylase and α-glucosidase).

No	Compounds name	RT (min)	RIE/RIT	EO Area %	Extract 1 Area %	Extract 2 Area%	Docking scores[a] kKcal/mol	Docking scores[b] kcal/mol
01	Tricyclene[a]	8.19	916/921	0.21	–	–	–5.4	–5.7
02	α-Thujene[a]	8.41	921/924	0.04	–	–	–5.9	–6.2
03	α-Pinene[a]	8.78	931/932	76.03	13.01	16.21	–5.4	–5.9
04	Camphene[a]	9.2	941/942	0.72	0.07	0.09	–5.5	–4.6
05	Thuja-2,4(10)-diene[a]	9.42	947/953	0.30	–	–	–5.9	–6.2
06	Sabinen[a]	10.202	967/969	0.16	–	–	–5.9	–6.3
07	β-Pinene[a]	10.31	969/974	2.75	0.67	0.75	–5.4	–5.8
08	Myrcene[a]	10.94	985/988	1.73	0.37	–	–4.7	–5.7
09	3-Carene[a]	11.73	1004/1008	1.15	0.87	1.02	–5.6	–6.8
10	ρ-Cymene[a]	12.35	1018/1020	0.46	–	0.09	–5.9	–6.2
11	D-Limonene[a]	12.54	1022/1024	1.32	0.36	0.36	–5.7	–6.0
12	Terpinolene[a]	15.25	1082/1086	0.06	–	–	–5.6	–5.9
13	α-Pinene oxide[b]	15.66	1091/1099	0.1	–	–	–5.6	–4.7
14	Trans-Verbenol[b]	16.037	1120/1122	–	0.40	–	–5.8	–4.9
15	α-Campholenal[b]	16.95	1119/1122	0.38	0.40	0.17	–5.5	–5.0
16	Trans-Pinocrveol[b]	17.55	1132/1135	0.67	0.52	0.52	–5.9	–6.2
17	Cis-Verbenol[b]	17.65	1135/1137	0.06	0.27	0.79	–5.8	–6.2
18	Camphor[b]	17.75	1137/1141	0.82	–	–	–5.4	–4.6
19	p-Menth-3-en-8-ol[b]	18.01	1142/1145	0.09	–	–	–5.7	–6.3
20	Trans-Pinocamphone[b]	18.53	1154/1158	0.06	–	–	–5.7	–5.3
21	Borneol[b]	18.78	1159/1165	0.09	–	–	–5.8	–4.8
22	p-Mentha-1,5-dien-8-ol[b]	18.87	1161/1166	0.04	–	–	–6.1	–6.4
23	Terpinen-4-ol[b]	19.34	1171/1174	0.22	–	–	–5.8	–6.1
24	ρ-Cymen-8-ol[b]	19.71	1179/1176	0.11	–	–	–5.8	–6.3
25	α-Terpineol[b]	19.96	1185/1186	0.42	0.27	0.24	–5.8	–6.3
26	Myrtenol[b]	20.22	1190/1194	0.19	–	–	–5.6	–5.0
27	ρ-allyl- Anisole[e]	20.366	1193/1195	0.08	–	–	–5.5	–5.7
28	Verbenone[b]	20.79	1202/1204	0.14	–	–	–5.7	–5.6
29	Bornyl acetate[b]	24.323	1280/1254	0.25	–	–	–6.0	–5.3
30	Verbenyl acetate[b]	24.78	1292/1291	0.19	–	–	–6.0	–5.2
31	δ−Elemene[c]	26.63	1333/1135	0.07	–	–	–6.7	–6.0
32	α-Terpinyl acetate[b]	27.13	1344/1346	0.56	0.64	0.57	–6.2	–6.9
33	α-Copaene[c]	28.301	1371/1374	0.06	–	–	–7.1	–6.8
34	β−Elemene[c]	28.995	1387/1389	0.52	0.42	0.35	–6.8	–5.8
35	Cedrene[c]	29.407	1396/1410	0.21	–	–	–7.7	–5.6
36	Caryophyllene[c]	30.15	1414/1417	3.10	1.62	1.45	–7.4	–6.0
37	γ-Elemene[c]	30.73	1428/1434	0.10	0.18	0.14	–6.5	–5.4
38	α-Humulene[c]	31.563	1448/1452	1.25	0.75	0.68	–7.1	–6.0
39	γ-Muurolene[c]	32.51	1471/1478	0.30	0.22	0.22	–7.2	–7.1
40	Germacrene D[c]	32.698	1475/1484	1.70	1.05	0.74	–7.4	–6.4
41	β-Selinene[c]	32.89	1481/1489	0.17	0.14	0.10	–7.4	–6.2
42	trans-Muurola-4(15), 5-diene[c]	33.12	1486/1493	0.10	–	–	–6.9	–6.7
43	Valencene[c]	33.25	1489/1496	0.18	–	–	–7.1	–7.3
44	α-Muurolene[c]	33.47	1494/1500	0.30	0.26	0.20	–7.1	–5.7
45	δ-Amorphene[c]	34.02	1508/1511	0.13	–	–	–7.0	–6.7

(*continues*)

Table 3. Continued.

No	Compounds name	RT (min)	RIE/RIT	EO Area %	Extract 1 Area %	Extract 2 Area%	Docking scores[a] kKcal/mol	Docking scores[b] kcal/mol
46	Cubebol[c]			–	0.18	0.31	–7.4	–7.3
47	δ-Cadinene[c]	34.39	1518/1522	0.80	0.45	0.53	–7.0	–6.8
48	Elemol[d]	35.387	1543/1548	0.13	0.59	0.49	–6.7	–5.9
49	Germacrene B[d]	35.703	1551/1559	0.72	0.34	0.26	–7.4	–5.8
50	Caryophyllene oxide[d]	36.697	1577/1582	0.53	1.64	1.64	–7.1	–5.8
51	Salvial-4(14)-en-1-one[d]	37.140	1588/1594	–	0.17	0.12	–7.2	–5.5
52	Humulene epoxide II[d]	37.705	1603/1608	0.12	0.71	0.63	–7.5	–5.8
53	1-epi-Cubenol[d]	38.444	1623/1641	0.08	–	–	–7.0	–6.0
54	Isospathulenol[d]	38.45	1623/1630	–	0.71	–	–6.9	–6.3
55	Aromadendrene epoxide[d]	39.101	1640/1641	–	0.16	0.14	–9.1	–7.5
56	β-Eudesmol[d]	39.247	1644/1650	–	0.17	0.14	–8.5	–7.8
57	Eudesma-4,11-dien-2-ol[d]	39.36	1648/nd	–	1.19	0.99	–7.4	–6.5
58	Schyobunol[d]	40.737	1684/1688	–	0.24	0.16	–6.5	–5.1
59	3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)] decane-9,10-diol[e]	41.13	1695/1710	–	0.25	–	–8.9	–7.2
60	Oplopanone[d]	42.40	1731/1739	–	0.54	0.32	–6.9	–5.6
61	Acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxo butylidene) oxepan-2-yl ester[e]	43.47	1762/nd	–	–	0.18	–6.8	–5.8
62	α-Vetivol[d]	43.49	1762/nd	–	0.30	–	–7.5	–6.3
63	Platambin[d]	46.48	1848/nd	–	0.20	–	–6.8	–6.1
64	Manool oxide[g]	50.91	1984/1987	–	–	0.25	–8.1	–6.8
65	Abitatriene[f]	52.90	2047/2055	–	0.27	0.19	–9.0	–8.5
66	Abitadiene[f]	53.675	2071/2087	–	0.95	0.75	–9.0	–8.2
67	19-norabieta-8,11,13-triene[g]	56.41	2164/nd	–	0.89	0.70	–8.5	–7.2
68	4-Hydroxy-18 -nor-abietane[g]	56.60	2170/nd	–	0.27	–	–8.5	–7.8
69	Methyl abietate[g]	57.628	2205/2385	–	–	1.89	–8.4	–8.0
70	Sandaracopimarinal[g]	56.701	2174/2184	–	0.31	0.22	–8.4	–7.2
71	Palustrale[g]	57.846	2213/nd	–	–	0.88	–8.5	–7.5
72	Methyl levopimarate[g]	57.628	2205/2305	–	2.18	–	–8.7	–7.6
73	Levopimarinal[g]	58.051	2220/nd	–	0.37	0.88	–8.8	–7.5
74	Androst-5-ene-3, 17-diol, 3-acetate,(3.β.,17.β.)[e]	58.56	2239/nd	–	0.17	–	–10.0	–8.9
75	Dehydro Abietal[g]	58.854	2249/2274	–	0.70	0.72	–8.7	–7.9
76	3-α-hydroxy Manool[g]	59.015	2255/2297	–	1.03	0.90	–9.0	–8.2
77	Abieta-8,11,13-trien- 7-one[g]	59.1	2257/nd	–	0.63	–	–9.1	–8.0
78	Abietal[g]	59.788	2282/2313	–	10.75	13.22	–8.9	–7.9
79	Abieta-7,13-dien-3-one[g]	60.16	2295/nd	–	4.02	4.55	–8.9	–7.3
80	4-Epiabietol[g]	60.36	2302/2343	–	0.26	–	–8.5	–7.7
81	Verticiol[g]	60.66	2313/nd	–	0.58	–	–8.6	–6.4
82	Dihydro Abietol[g]	60.354	2302/2368	–	0.58	0.23	–8.7	–8.0
83	Neoabietyl acetate[g]	61.075	2328/nd	–	0.51	1.24	–9.0	–8.6
84	4-epi- dehydroabietol[g]	61.504	2344/nd	–	1.22	1.33	–8.5	–6.6
85	Communic acid[g]	61.81	2355/2365	–	23.66	22.38	–8.4	–7.3
86	Bis(2-methoxy phenyl) (isopropoxy) methane[e]	62.11	2366/nd	–	2.03	–	–6.5	–6.3

(*continues*)

Table 3. Continued.

No	Compounds name	RT (min)	RIE/RIT	EO Area %	Extract 1 Area %	Extract 2 Area%	Docking scores[a] kKcal/mol	Docking scores[b] kcal/mol
87	Abieta-8(14),13(15)-dien-18-al[f]	62.114	2367/nd	–	–	1.32	–8.9	–7.8
88	Abietol[g]	62.447	2379/2401	–	4.25	4.60	–8.8	–7.8
89	16-Norisopimar-7-en- 15-ol[g]	63.1	2402/nd	–	0.83	–	–8.6	–6.9
90	Abietic acid[g]	63.798	2430/nd	–	–	1.95	–8.9	–7.8
91	Abietate[g]	63.87	2433/nd	–	1.07	–	–8.9	–7.9
92	Neoabietol[g]	64.267	2448/2468	–	–	0.25	–9.2	–8.2
93	Methyl tetratriacontyl ether[e]	79.21	3084/nd	–	0.32	0.45	–5.7	–5.4
94	Anthricin[e]	82.347	3237/nd	–	0.51	0.25	–8.0	–7.6
95	Tetrapentacontane[e]	83.12	3275/nd	–	0.15	–	–5.2	–5.7
96	γ-Sitosterol[é]	83.50	3295/nd	–	0.22	0.36	–9.3	–8.5

Monoterpene hydrocarbons – 23.52%; 6.74%; 7.31%
Oxygenated monoterpens – 33.33%; 6.74%; 6.09%
Sesqueterpene hydrocarbons – 29.41%; 10.11%; 12.19%
Oxygenated sesqueterpens – 9.80%; 14.60%; 12.19%
Non-terpene derivative – 1.96%; 6.74%; 4.87%
Diterpene hydrocarbons – 2.25%; 3.65%
Oxygenated diterpenes –21.34%; 21.95%
Totale identified (%) – 98%; 67%; 68%
Not identified (%) – 2%; 32%; 32%
EO: Essential oil; **nd**: non–determinated; **E**: Experimentale; **T**: Theorical;**1**: Cyclohexane extract; **2**: Ethyl acetate extract; **a**: α-amylase docking scores; **b**: α-glucosidase docking scores.

and ester (Acetic acid, 2,6,6-trimethyl-3-methylene-7-(3-oxobutylidene)oxepan-2-yl ester). For both extracts (cyclohexane and ethyl acetate), the main components were derived from the oxygenated diterpenes such as communic acid (23.66% and 22.38%), abitale (110.75% and 13.22%), abitole (4.25% and 4.60%), and abieta-7,13-diene-3-one (4.02% and 4.55%), respectively. Despite the existence of α-pinene with significant amounts for both extracts with percentages of 13.01% and 16.21 %, which proves the presence of *J. phoenicea*. The GC-MS analysis of the non-polar extracts reveals chemical variations in the content compared with the EO, noting the presence of diterpene hydrocarbons, oxygenated diterpenes in significant amounts, and sesquiterpene oxygen, which is present in significant quantities as in the EO. The total ion chromatogram of representative samples in which the main compounds are pointed out is displayed in Figure 1. The complete EO composition is shown in Table 2.

The preliminary study examined the chemical composition, antioxidants, antidiabetic effects, and *in silico* modeling of the EO and non-polar extracts of Algerian *J. phoenicea* berries. The results of this study showed that the EO was characterized by α-pinene, caryophyllene, β-pinene, myrcene, and germacrene D. These results, although slightly different in profile from earlier research, are generally consistent with previous findings (Amalich *et al*., 2015; Angioni *et al*., 2003; Medini *et al*., 2011; Menaceur *et al*., 2013, Abdelli et al. 2018). The minor differences in EO composition could be attributed to genotype-environment interactions and the phenological stage of the species under study. Plants may produce certain chemical biomolecules as an adaptive response to changing environmental conditions. The results also indicated that the principal elements mentioned above were present in the non-polar extracts. While there were no previous studies mentioning the composition of non-polar extracts, especially for cyclohexane and ethyl acetate, the current outcome was consistent with the literature. The main constituents of most *J. phoenicea* species include abitale, abitole abieta-7,13-diene-3-one, and α-pinene (Boudiba *et al*., 2021 Bouyahyaoui *et al*., 2016; Ennajar *et al*., 2010).

According to literature data, extracts such as ethyl acetate, ethanol, and methanol solvents had the highest content of total phenols and flavonoids (Bouyahyaoui *et al*., 2016). This could be explained by the fact that polar solvents have a higher affinity for these components than non-polar solvents. On the other hand, variations in phenolic content might be attributed to different extraction methods, the standard solution used, geographical location, and climate conditions. Overall, the study reported that total phenolic and total flavonoids levels were relatively low.

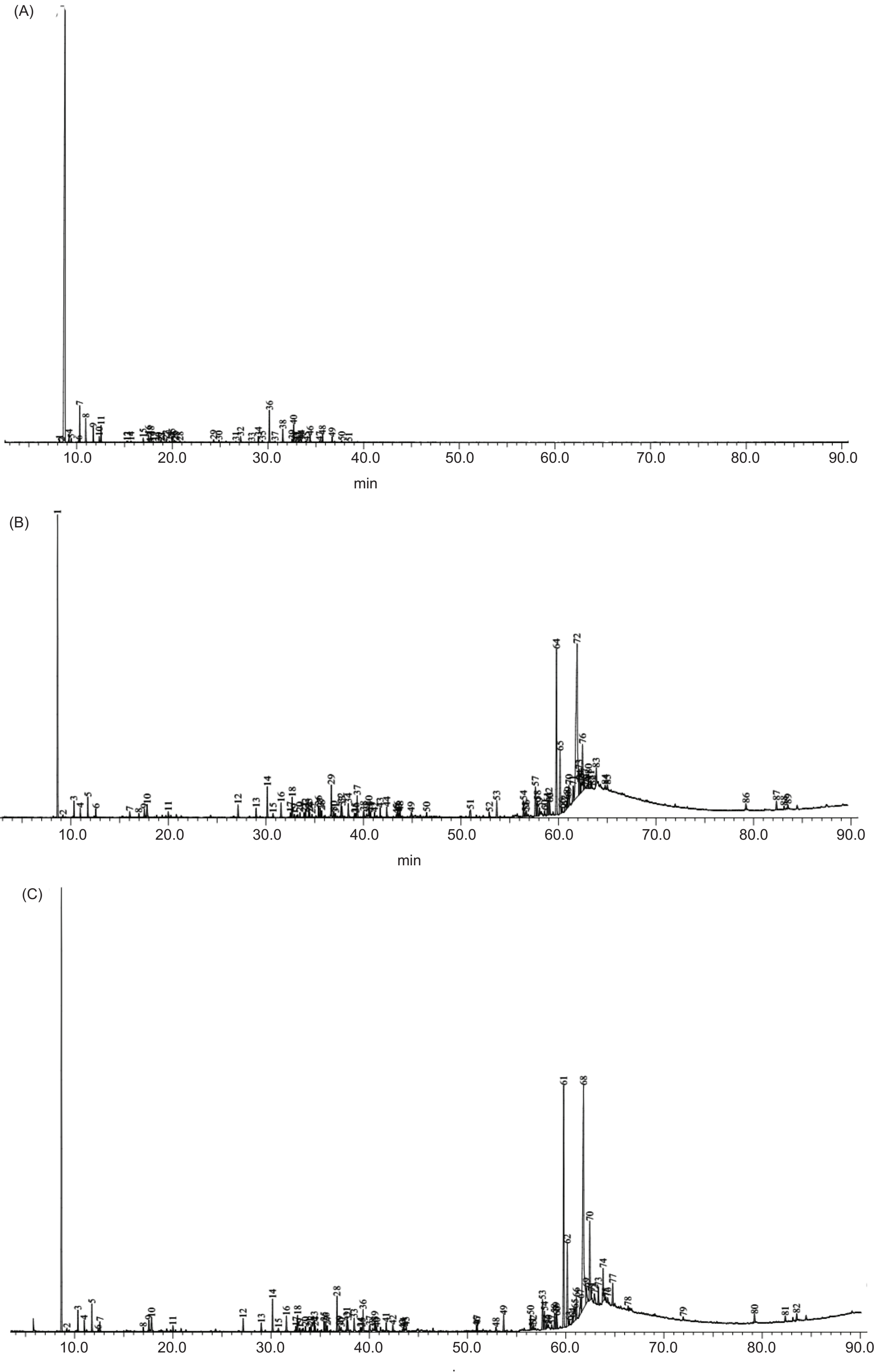

Figure 1. GC-MS total ion chromatogrammes from a representative sample of berries of *Juniperus phoenicea* L. (A) Essential oil. (B) Cyclohexane extract. (C) Ethyl acetate extract.

Antioxidant activities

A total of three *in vitro* antioxidant tests (DPPH, ABTS, and phenanthroline) were conducted to compare with five standards: BHA, BHT, ascorbic acid, quercetin, and α-tocopherol. The results were obtained using linear regression analysis, and the IC_{50} and $A_{0.5}$ values are presented in Table 3 as the mean values ± SD of three measurements. In most tests, the IC_{50} and $A_{0.50}$ values differed significantly from the standard ones.

Antioxidants operate through two distinct processes: electron transfer (single electron transfer, SET) and hydrogen donation (hydrogen atom transfer, or HAT). Our investigation utilized several techniques ($DPPH^{•}$, $ABTS^{•+}$, and phenanthroline) to gather more data on the antioxidant activity of the examined extracts. The differences between these tests lie in their modes of action and reaction mechanisms.

In the DPPH assay, all samples showed lower activity (IC_{50} > 800 g/mL) compared to the standards. The lower activity exhibited by all samples was likely due to the high concentration of terpene hydrocarbons (53%) in the EO. Similarly, the non-polar extracts showed lower activity despite containing a high concentration of oxygenated terpene (40.23%–42.68%). This can be attributed to the fact that the inhibitory activity of the DPPH radical is not solely dependent on the total polyphenol content, but rather on specific polyphenols with unique chemical structures (Sánchez-Vioque *et al.*, 2013). In other words, DPPH is highly selective and only reacts with flavonoids that contain hydroxyl groups in the B cycle (Yokozawa *et al.*, 1998), as well as aromatic acids with multiple hydroxyl groups, which were not observed in our case. These results were in agreement with existing literature on similar species, such as the antioxidant activity studies conducted by Ennajar *et al.* (2009) and Taviano *et al.* (2011) on methanol and water extracts of five different Turkish *Juniperus* species, which showed weaker activity compared to the extracts examined in this study (Ennajar *et al.*, 2009; Taviano *et al.*, 2011). On the other hand, compared to the polar extract case by Ghouti *et al.* (2018) where hydroethanolic and infusion extracts were used, our study demonstrated interesting DPPH activity that was two to three times more effective than Trolox. Additionally, research by Keskes *et al.* (2014) reported that the methanol extract exhibited a stronger ability to scavenge DPPH radicals (EC_{50} value = 2 μg/mL).

Regarding the phenanthroline assay, the EO and non-polar extracts of *J. phoenicea* showed a weak ability to reduce iron with $A_{0.50}$ value of >200 μg/mL for the high concentration. This is in contrast to the results of standards such as ascorbic acid, quercetin, BHA, BHT, and α-tocopherol, which demonstrated a substantial reducing impact on iron. Our results indicated a low absorbance for both extracts (200 μg/L – 0.35), which is comparable to findings in the literature (Byrne *et al.*, 2016; Menaceur *et al.*, 2013). The phenolic content of the extracts was closely correlated with their poor activity in the DPPH radical scavenging and phenanthroline tests. Numerous investigations have shown a strong positive association between total phenolic content, phenanthroline, and anti-DPPH action.

In the ABTS assay, it was observed that the extracts of *J. phoenicea* berries exhibited a stronger free radical scavenging capacity compared to DPPH for all samples. This can be attributed to the versatility of the radical cation ABTS, which is soluble in both water and organic solvents. This allows evaluation of anti-radical activity for both hydrophilic and lipophilic compounds. Additionally, the planar form of ABTS makes it more susceptible to attack compared to DPPH, which is more complex (Gülçin, 2010). When compared to the five positive standards, ABTS still showed minimal activity. The percentage of ABTS-scavenging ability of the EO from Tunisian *J. pheonicea* berries was determined to be 128.7 ± 3.8 μg/mL compared to vitamin C at 1.9 ± 0.9 μg/mL. These results were slightly lower than previous investigations. Despite their low concentration, no correlation was found between phenolic compounds and $ABTS^{•+}$ activity. Certain monoterpene alcohols, ethers, ketones, aldehydes, and non-polar bioactive substances like pigments, were associated with $ABTS^{•+}$ activity (Edris, 2007; Merola *et al.*, 2017).

In general, the antioxidant capacity of both EO and non-polar extracts (cyclohexane and ethyl acetate) of *J. phoenicea* berries was tested in three different assays (DPPH, ABTS, and phenanthroline). It can be concluded that the EO exhibits no antioxidant activity despite increases in concentration. This could be explained by the complex mixture of EOs that may exhibit antioxidant activity different from that of their major components tested alone. Literature reports suggest that the antioxidant activity of EO may result from a complex interaction between different constituents, producing additive, synergistic, or antagonistic effects, even at low concentrations (Ennajar *et al.*, 2010). The non-polar extracts show some antioxidant activities but are still insignificant compared to five standards. This could be due to the low phenolic content and the mixture of extracts preventing the reduction of both DPPH and ABTS, and even forming a complex with phenanthroline for reducing the ferric ion. Table 3 presents the different values obtained in the antioxidant activities.

Anti-diabetic activity

One of the most crucial approaches to treating type 2 diabetes (T2D) is controlling postprandial hyperglycemia.

This is achieved by inhibiting the enzymes that hydrolyze carbohydrates, primarily intestinal α-glucosidase (EC 3.2.1.21.), and pancreatic α-amylase (EC 3.2.1.1.1.). This process may reduce blood levels of glucose in circulation by delaying the gastrointestinal absorption of dietary carbohydrates (Al-Ishaq *et al.*, 2019; Deo *et al.*, 2016; Priscilla *et al.*, 2014). Due to these factors, the present investigation examined our plant's potential as an antidiabetic by evaluating its inhibitory effect on the enzymatic activity of α-amylase. The primary enzyme in humans, α-amylase, breaks down starch into simple sugars such as dextrin, maltotriose, maltose, and glucose. α-amylase inhibitors are believed to enhance glucose tolerance in diabetic patients, although the activity of the enzyme has not been directly linked to the genesis of diabetes. To improve diabetes management, great effort has been put forward in recent years to discover α-amylase inhibitors that are clinically effective (Loizzo *et al.*, 2007). The results are expressed as mean ± SD and provided in Table 4.

Table 4. Anti-diabetic activities (α-amylase) of *Juniperus phoenicea* berries essential oil and non-polar extracts.

Extracts/standard	α-amylase	
	Max inhibitory %	IC_{50} (μg/mL)
Essential oil	33.47 ± 2.58	>1600^{d}
Cyclohexane	69.59 ± 0.07	186.91 ± 5.74^{d}
Ethyl acetate	88.71 ± 0.47	351.48 ± 0.17^{d}
Acarbose	53.05 ± 1.6	3650.9 ± 1.7^{d}

Inhibition rates and IC_{50} values are expressed as means ±S.D (n = 3). Mean values followed by different letters are significantly different (based on one-way ANOVA followed by Tukey's multiple comparison tests, Level of Significance: p < 0.0001.

As demonstrated by the results obtained, both extracts showed a strong dose-dependent inhibitory effect against α-amylase, with maximum inhibition rates >75.0% at the high tested dose of 400 μg/mL. Table 4 shows the significant increase in the α-amylase inhibitory effect with an IC_{50} of 186.91 ± 5.74 μg/mLfor the cyclohexane extract compared to 351.48 ± 0.17 μg/mL for the ethyl acetate extract. This difference is nearly 20 times greater than that of acarbose, used as a positive control. Interestingly, acarbose's inhibitory efficacy against α-amylase is about 15 times less (3650.9 ± 1.7 μg/mL) than the ethyl acetate extract. However, pure quercetin demonstrated effective inhibition of α-amylase with the lowest IC_{50} value of 4.3 ± 0.2 μg/mL, surpassing the activity observed in our investigation against α-amylase. These results suggest the strong anti-diabetic properties of both non-polar extracts (cyclohexane and ethyl acetate) of *J. phoenicea* berries. In contrast, the EO showed a maximum inhibition of 33% at a concentration of 16 mg/L, providing a reliable value compared to the extracts.

Regrettably, the literature lacked significant information about the potential inhibitory effects of *Juniperus* species against α-amylase, while few reports on *J. phoenicea* berries' α-amylase inhibitory activities were published. Interestingly, Loizzo *et al.* (2007) investigated the α-amylase inhibitory activities of *J. oxycedrus* berries EO. The bio-assay was adopted and modified based on the work of Conforti *et al.* (2005). Their results indicated moderate activities of berries EO with IC_{50} value of >25 μL/mL (Loizzo *et al.*, 2007). In the same context, the work of Orhan *et al.* (2012) evaluated the hypoglycemic and antidiabetic activity of *J. oxycedrus* subsp. *Oxycedrus* berries. They studied the oral administration of berry extracts using *in vivo* models in normal and glucose-hyperglycemic rats. They indicated that ethanol extract showed a higher and continuous hypoglycemic effect (7.7%–23.6%) in STZ-diabetic rats. For normal rats, the ethanol extract exhibited a moderate hypoglycemic effect (12.8%–13.0%). The n-ButOH extract gives better results, especially for fraction C, which exhibited the most effective antidiabetic activity compared with others between 18.6% and 26.4%. This activity was caused by the presence of bioactive compounds such as shikimic acid (Orhan *et al.*, 2012).

Banerjee *et al.* (2013) studied the impact of a methanolic extract of *J. communis* (100 mg/kg, 200 mg/kg) administered orally to streptozotocin nicotinamide-induced diabetic rats. They found that the extract had dose-dependent and significant anti-diabetic and antihyperlipidemic properties, supporting its potential as an effective drug for type-2 diabetes. In another study conducted by Sánchez de Medina *et al.* (1994) and Swanston-Flatt *et al.* (1990), it was shown that decoction and infusion of *J. communis* fruits led to significant decrease in blood glucose levels in both normoglycemic rats and STZ-induced diabetic rats and mice.

Overall, research on the antidiabetic effects of *Juniperus* species, both *in vitro* and *in vivo*, suggests strong inhibitory activity against α-amylase and α-glucosidase. These findings have the potential to improve hyperglycemia in individuals with type 2 diabetics, highlighting the need for further investigation into the mechanisms of action and active components of these plants.

Molecular docking

Molecular docking is an increasingly common method in structure-based drug design (SBDD) because it can accurately predict the binding conformation of small molecules on their corresponding target proteins

(Wang *et al*., 2020). This technique facilitates the analysis and visualization of significant intermolecular biological processes, including the binding of ligands to their corresponding receptors and interactions that maintain the stability of the ligand-receptor system. Furthermore, it is possible to rank the "docked" conformations of the simulated ligands by using their binding free energies since molecular docking techniques quantify receptor-ligand binding energies (Huang and Zou, 2010; Kadi *et al*., 2023).

To understand the binding efficacy of compounds from *J. phoenicea* L. berries' EOl and non-polar extracts (cyclohexane and ethyl acetate) to the selected proteins associated with diabetes (α-amylase 4W93 and α-glucosidase 3W37), molecular docking simulations were conducted using Autodock Vina. The native ligands montbretin A and acarbose were used for comparison, and their binding energies were -11.0 and -10.0 kcal/mol, respectively. Among those results, binding energy values varied from -4.6 to -10.0 kcal/mol. The best result in this range was measured in terms of the score value of the native ligand, and the three best compounds from each extract composition were selected.

Our findings showed potent molecules such as Humulene epoxide II, Caryophyllene, and cedrene against α-amylase, valencene, γ-muurolene, and δ-cadinene for α-glucosidase in the EO composition. In the cyclohexane extract, the most potent ones were androst-5-ene-3,17-diol, 3-acetate (3.β.,17.β.), γ-sitosterol and abieta-8,11,13-trien-7-one, for α-amylase and androst-5-ene-3,17-diol,3-acetate (3.β.,17.β.), neoabietyl acetate, and γ-Sitosterol for α-glucosidase. In the ethyl acetate extract, γ-Sitosterol, Neoabietol, and Aromadendrene epoxide showed a potent affinity against α-amylase while abitatriene, γ-sitosterol, and neoabietol for α-glucosidase.

Major compounds for each extract showed good results but this varied for each protein, possibly due to the amino acid construction of each protein. Although there have been fluctuations in this range, the result of diterpene and sesquiterpene is still high compared to monoterpene, reflecting the fact that their structure has many hydroxyl groups that facilitate ligands in forming hydrogen bonds with a free residue of the receptor. Additionally, Table 4 shows the best receptor of these bioactive compounds in *J. phoenicea* L. berries, α-amylase 4W93 followed by α-glucosidase 3W37. Interaction analysis in 3D and 2D images was performed to understand the binding mode of the studied compounds in interaction with α-amylase 4W93 and α-glucosidase 3W37. Figures 2 and 3 show the different interactions.

Our results showed that in the case of ethyl acetate extract, γ-Sitosterol is the most interactive ligand with α-amylase 4W93. This interaction is facilitated through two positive ionizable interactions formed with the catalytic triad of hydrogen-donor, with ASP197 and GLU233, five interactions (one π-sigma and four π-alkyl) with TRP59, and one alkyl interaction observed with LEU162. On the other hand, in the case of α-glucosidase 3W37, the most interactive ligand was abitatriene. This interaction involved three interactions with the first ring, one π-anion with ASP568, one intriguing π-sulfur interaction with MET470, and one π-π T-shaped interaction with TRP432.

For the cyclohexane extract, the most interactive ligand in this study, for α-amylase was androst-5-ene-3,17-diol,3-acetate (3.β.,17.β.). It showed two hydrogen donors intracting with HIS201 and GLN63, two π-π interactions with TRP59, and three hydrophobic alkyl interactions, two with ALA198, and one with LEU162. In the case of EO, we observed lower values for α-amylase activity, which was confirmed by molecular docking. Lower values were also registered for both proteins α-amylase 4W93 and α-glucosidase 3W37. Cedrene was identified as the best compound with the highest binding energy with α-amylase, forming two π-sigma interactions with TYR62 and TRP59, and two π-alkyl interactions with TRP58 and TYP92. On the other hand, valencene showed better binding energy with α-glucosidase 3W37, with four interactions with the same amino acid TRP59, two being π-sigma and two being π-alkyl. Table 5 summarizes the different values of binding energies, type, and distances of each interaction for both proteins α-amylase 4W93 and α-glucosidase 3W37.

Human pancreatic α-amylase is 56 kD protein composed of 496 amino acids as single peptide chains divided into three domains: domain A (residues 1–99 and 169–404), the largest part where the active site is located as a V-shaped cleft in the catalytic domain A that binds to the substrate, domain B (residues 100–168) emerges from domain A and is located between the third β-strand and the α-helix of the β-barrel of the catalytic domain, maintaining the conformation of the enzyme, and domain C (residues 405–496) is an anti-parallel β-sheet of domain A loosely associated with other domains (Brayer *et al*., 1995; Mótyán *et al*., 2011; Nahoum *et al*., 2000; Williams *et al*., 2012). According to the results of our research, domain A accounts for the majority of contacts with a particular level of efficacy such as those of ASP197 and GLU233 except for the interaction of LEU162 which belongs to domain B and presents a lower hydrophobic interaction.

In the same context, the structures of α-glucosidases are complex. There are four primary domains in this structure: two C-terminal domains, a catalytic domain of the (β/α) 8-barrel, and an N-terminal domain. The inserts 1

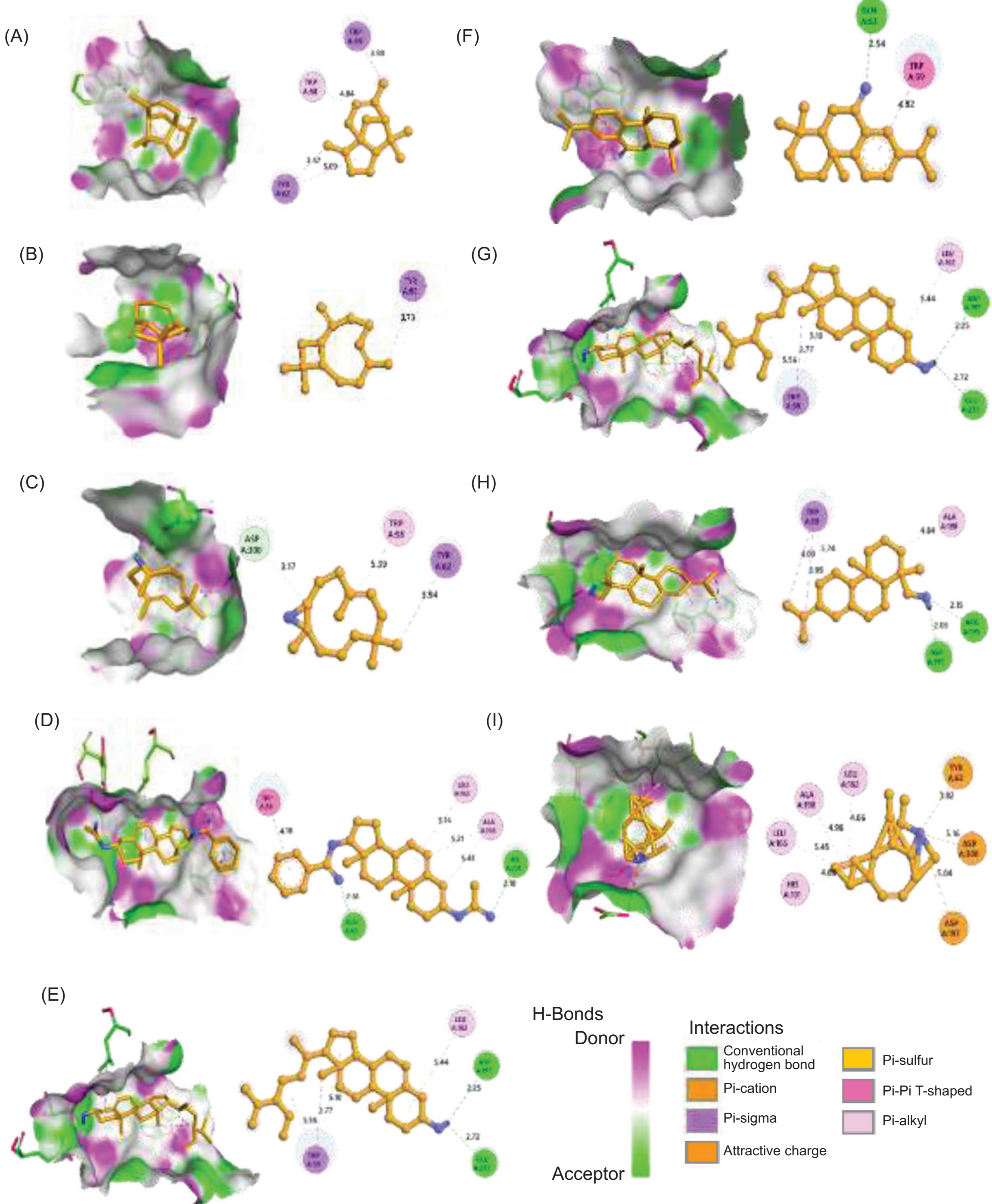

Figure 2. The molecular interactions in 2D and 3D images between the studied compounds and the main α-amylase 3W93 using Autodock Vina. Essentail oil: (A) Cedrene, (B) Caryophyllene, (C) Humulene epoxide II. Cyclohexane extract: (D) Androst-5-ene-3,17-diol, 3-acetate, (E) g-Sitosterol, (F) Abieta-8,11,13-trien-7-one. Ethyl acetate extract: (G) g-Sitosterol, (H) Neoabietol, (I) Aromadendrene epoxide.

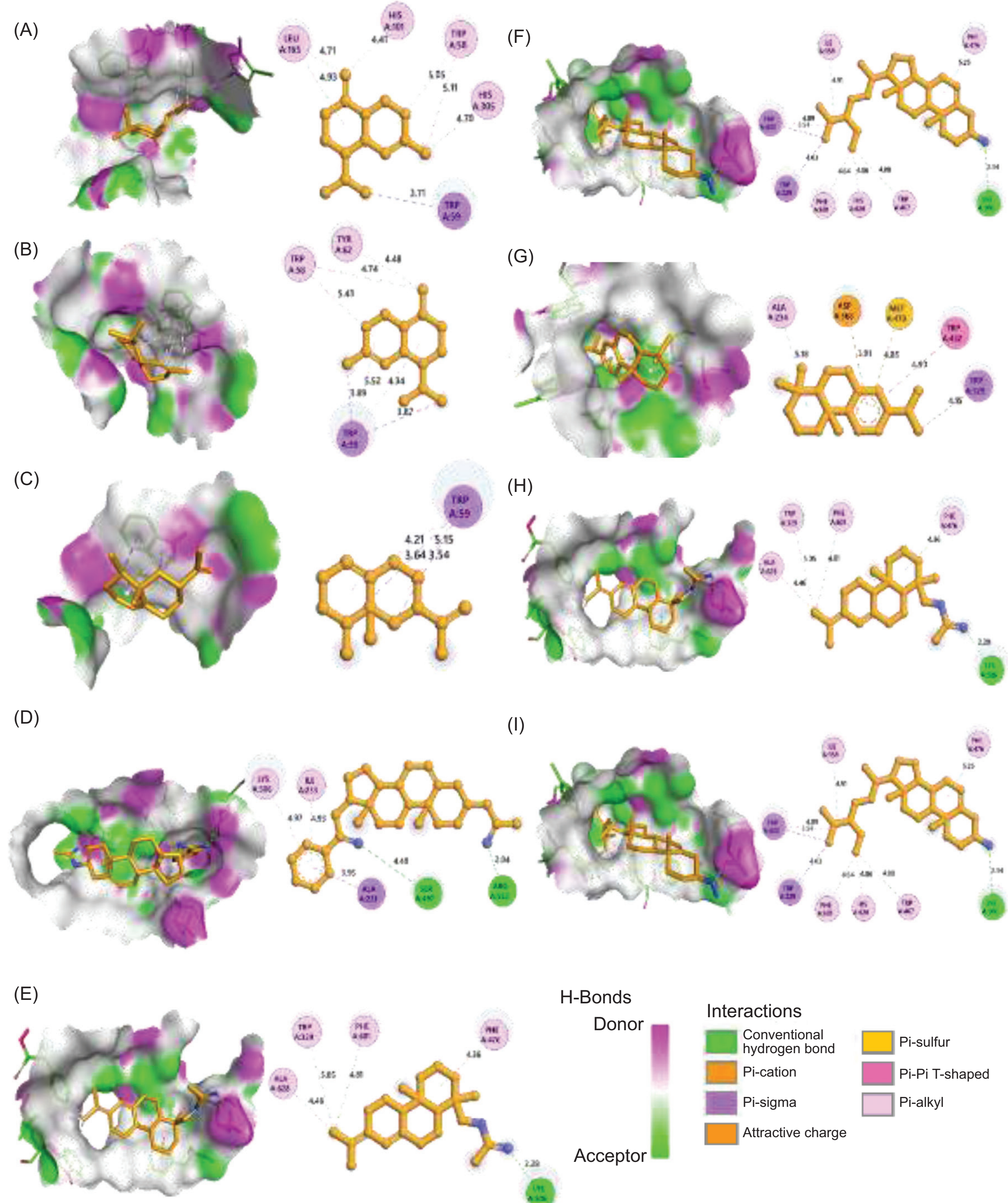

Figure 3. The molecular interactions in 2D and 3D images between the studied compounds and the main α-glucosidase 4W37 using Autodock Vina. Essential oil: (A) γ-Muurolene, (B) δ-Cadinene, (C) Valencene. Cyclohexane extract : (D) Androst-5-ene-3,17-diol, 3-acetate, (E) Neoabietyl acetate, (F) γ-Sitosterol. Ethyl acetate extract: (G) Abitatriene, (H) Neoabietyl acetate, (I) γ-Sitosterol.

Table 5. Different values of banding energies, type and distances of each interaction for both proteins α-amylase 4W93 and α-glucosidase 3W37.

Proteins	Sample	Molecules	Structures	Banding energy kcal/mol	Interacting amino acid residue	Distance between interacting residue (A°)	Type of bond
α-amylase 4W93	**Essential Oil**	Cedrene		–7.7	TYR62 TRP59 TRP58 TYR62	3,56675 3,98121 4,83743 5,09309	π-Sigma π-Sigma π-Alkyl π-Alkyl
		Humulene epoxide II		–7.5	ASP300 TYR62 TRP58	3,17348 3,93668 5,38633	H-Donor π-Sigma π-Alkyl
		Caryophyllene		–7.4	TYR62	3.72568	π-Sigma
	Cyclohexane extract	Androst-5-ene-3, 17-diol, 3-acetate, (3.β.,17.β.)		–10	GLN63 HIS201 TRP59 TRP59 LEU162 ALA198 ALA198	2,61452 2,09628 3,87683 3,7666 5,1361 5,21196 5,4258	H-Donor H-Donor π-π π-π Alkyl Alkyl Alkyl

(*continues*)

Table 5. Continued.

Proteins	Sample	Molecules	Structures	Banding energy kcal/mol	Interacting amino acid residue	Distance between interacting residue (A°)	Type of bond
		γ-Sitosterol		–9.3	ASP197 GLU233 TRP59 LEU162 TRP59 TRP59 TRP59 TRP59	2,24848 2,72316 3,77219 5,43775 4,48837 4,33389 4,63034 4,7324	H-Donor H-Donor π-Sigma Alkyl π-Alkyl π-Alkyl π-Alkyl π-Alkyl
		Abieta-8,11,13-trien-7-one		–9.1	GLN63 TRP59 TRP59	2,54073 4,14617 4,73227	H-Donor π-π π-π
	Ethyl acetate extract	γ-Sitosterol		–9.3	ASP197 GLU233 TRP59 LEU162 TRP59 TRP59 TRP59 TRP59	2,24848 2,72316 3,77219 5,43775 4,48837 4,33389 4,63034 4,7324	H-Donor H-Donor π-Sigma Alkyl π-Alkyl π-Alkyl π-Alkyl π-Alkyl
		Neoabietol		–9.2	ARG195 ASP197 TRP59 TRP59 ALA198 TRP59 TRP59	2,14733 2,02872 3,99979 3,99367 4,84297 4,87094 5,07496	H-Donor H-Donor π-Sigma π-Sigma Alkyl π-Alkyl π-Alkyl

		Aromadendrene epoxide	–9.1	ASP197	5,04058	Attr-charge
				ASP300	5,16234	Attr- charge
				TYR62	3,81917	π-cation
				ALA198	4,9616	Alkyl
				LEU162	5,0903	Alkyl
				LEU165	5,45147	Alkyl
				HIS101	4,69396	π-Alkyl
α-glucosidase 3W37	**Essential oil Cyclohexane extract**	Valencene	–7.3	TRP59	3,63801	π-Sigma
				TRP59	3,54015	π-Alkyl
				TRP59	4,21244	π-Sigma
				TRP59	5,15059	π-Alkyl
		γ-Muurolene	–7.1	TRP59	3,71486	π-Sigma
				LEU165	4,92721	Alkyl
				LEU165	4,70831	Alkyl
				TRP58	5,05238	π-Alkyl
				TRP58	5,10866	π-Alkyl
				HIS101	4,40532	π-Alkyl
				HIS305	4,70065	π-Alkyl
		δ-Cadinene	–6.8	TRP59	3,89377	π-Sigma
				TRP59	3,87039	π-Sigma
				TRP58	5,42743	π-Alkyl
				TRP58	4,74245	π-Alkyl
				TRP59	4,90992	π-Alkyl
				TRP59	4,20798	π-Alkyl
				TRP59	5,34186	π-Alkyl
				TRP59	5,412	π-Alkyl
				TRP59	5,04359	π-Alkyl
				TYR62	4,47548	π-Alkyl
		Androst-5-ene-3, 17-diol, 3-acetate,(3.β.,17.β.)	–8.9	SER497	2,2528	H-Donor
				SER497	2,21715	H-Donor
				ARG552	2,03861	H-Donor
				ALA231	3,94692	π-Sigma
				ILE233	4,93109	π-Alkyl
				LYS506	4,97178	π-Alkyl

(continues)

Table 5. Continued.

Proteins	Sample	Molecules	Structures	Banding energy kcal/mol	Interacting amino acid residue	Distance between interacting residue (A°)	Type of bond
		Neoabietyl acetate		–8.6	LYS506	2,28774	H-Donor
					ALA628	4,456	Alkyl
					TRP329	5,05483	π-Alkyl
					PHE476	4,3611	π-Alkyl
					PHE601	4,80502	π-Alkyl
		γ-Sitosterol		–8.5	LYS506	2,34471	H-Donor
					TRP329	3,59918	π-Sigma
					TRP329	3,44091	π-Sigma
					TRP432	3,96979	π-Sigma
					ILE358	4,90631	Alkyl
					TRP432	4,89168	π-Alkyl
					TRP467	4,98388	π-Alkyl
					PHE476	5,24734	π-Alkyl
					PHE601	4,63809	π-Alkyl
					HIS626	4,85521	π-Alkyl
	Ethyl acetate Extract	Abitatriene		–9.0	ASP568	3,91321	π-Anion
					TRP329	3,82669	π-Sigma
					TRP329	3,88644	π-Sigma
					MET470	4,84509	π-Sulfur
					TRP432	4,90409	π-p
					ALA234	5.1829	alkyl
		Neoabietyl acetate		–8.6	LYS506	2,28774	H-Donor
					ALA628	4,456	Alkyl
					TRP329	5,05483	π-Alkyl
					PHE476	4,3611	π-Alkyl
					PHE601	4,80502	π-Alkyl

γ-Sitosterol	−8.5	LYS506	2,34471	H-Donor
		TRP329	3,59918	π-Sigma
		TRP329	3,44091	π-Sigma
		TRP432	3,96979	π-Sigma
		ILE358	4,90631	Alkyl
		TRP432	4,89168	π-Alkyl
		TRP467	4,98388	π-Alkyl
		PHE476	5,24734	π-Alkyl
		PHE601	4,63809	π-Alkyl
		HIS626	4,85521	π-Alkyl

and 2 of the catalytic domain are situated next to β-stands 3 and 4, respectively. Except for insert 1, the overall architectures of these subunits are nearly identical. The β-barrel loops generate the active site pocket (Subsite-1) in the catalytic domain, and the residues involved in subsite-1 formation have been extensively conserved throughout the subunits of α-glucosidases (Kashtoh and Baek, 2022). In our study, the most interesting interaction that contributes to enzyme/ligand binding was established with SER497, ARG552, and LYS506, which provide hydrogen-donor interactions in the case of Androst-5-ene-3, 17-diol, 3-acetate, (3.β.,17.β.), Neoabietyl acetate, and γ-Sitosterol.

The compounds (best docked) from each extract were further investigated using the online software ADMETSAR, which provides more knowledge about pharmacokinetic, physiochemical behavior, and druglikeness. ADMET properties of those bioactive compounds, which include Caco-2 cell permeability, brain/blood barrier, human intestinal absorption, AMES mutagenesis, and carcinogenicity, were elucidated in the present study. Results of ADMETSAR were analyzed and tabulated in Tables 6 and 7. The molecular weight of all the bioactive compounds was found to be less than 500, anticipating their easy transportation, absorption, and diffusion. Hydrogen bonding describes drug permeability; poor permeation correlates to more than 5 H-bond donors and 10 H-bond acceptors, which were not detected in our results, indicating the best permeation for all tested compounds. Despite that, not all our tested compounds are in the range of acceptable ALog P value (≤ 5), but still orally absorbed based on the Lipinsky rule, an important consideration of the drug's likeness. Furthermore, a drug molecule is expected to be in an aqueous solubility range of –1 to –5, and the Log S values of all the selected compounds fall within the range except for the case of Abitatriene, which was observed with a value of -6.20 (Bergenhem, 2011). The different results are shown in Table 6. The determination of pharmacological qualities is crucial to identify bioactive molecules with acceptable pharmaceutical properties and then address their drug availability. This involves researching absorption, distribution, metabolism, excretion, and toxicity (Ghannay *et al*., 2020).

ADMET pharmacokinetic properties reveal that all selected compounds had better Human Intestinal Adsorption (HIA) with a score of > 99%. Greater HIA indicates that the compound could be better absorbed from the intestinal tract upon oral administration. In addition to the HIA, all selected compounds had a positive value of Caco-2 permeability and blood-brain barrier BBB. It is observed that the lower value is registered for both γ-Sitosterol and Androst-5 -ene-3,17-diol, 3-acetate,(3.β.,17.β.). The admetSAR online tool was used to evaluate the toxicological properties of the selected compounds, as

Table 6. Physicochemical parameters (Lipinski Rule of Five) of the best docked bioactive compounds of *Juniperus phoenicea* berries.

Molecules	Molecular weight	Number of HBA	Number of HBD	Number of Rotation Bond	ALog P	Log S
Cedrene	204.36	0	0	0	4.42	–4.80
Humulene epoxyde II	220.36	1	0	0	4.25	–3.18
Caryophyllene	204.36	0	0	0	4.73	–4.69
Valencene	204.36	0	0	1	4.73	–5.36
γ-Muurolene	204.36	0	0	1	4.58	–5.37
δ-Cadinene	204.36	0	0	1	4.73	–5.25
Androst-5-ene-3,17-diol, 3-acetate ,(3.β.,17.β.)	332.48	3	1	1	4.24	–5.20
γ-Sitosterol	414.72	1	1	6	8.02	–4.70
Abieta-8,11,13-trien-7-one	284.44	1	0	1	5.48	–4.52
Neoabietol	288.47	1	1	1	5.26	–3.95
Aromadendrene epoxide	220.36	1	0	0	3.48	–2.98
Neoabietyl acetate	330.51	2	0	3	5.68	–4.88
Abitatriene	270.46	0	0	1	5.84	–6.20

HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; ALog P: compound octanol/water partition coefficient; Log S: solubility coefficient.

Table 7. Pharmacokinetic ADMET profile of the best-docked bioactive compounds of *Juniperus phoenicea* berries.

Molecules	HIA[a]	Caco-2[b]	BBB[c]	HOB[d]	Carcinogenicity	Ames	Respiratory
	Mutagenesis toxicity						
	V P	V P	V P	V P	V P	V P	V P
Cedrene	+ 0.9930	+ 0.7991	+ 0.9750	+ 0.6000	– 0.7500	– 0.8900	+ 0.5222
Humulene epoxyde II	+ 0.9923	+ 0.8466	+ 0.9250	+ 0.7286	– 0.6900	– 0.7400	+ 0.5778
Caryophyllene	+ 0.9881	+ 0.8656	+ 0.9250	+ 0.6429	– 0.6500	– 1.0000	– 0.5556
Valencene	+ 0.9930	+ 0.9131	+ 0.9000	– 0.5714	– 0.7900	– 0.8500	– 0.7444
γ-Muurolene	+ 0.9946	+ 0.9185	+ 0.9250	+ 0.6571	– 0.7700	– 0.8400	– 0.6771
δ-Cadinene	+ 0.9958	+ 0.9654	+ 0.9250	+ 0.7000	– 0.8000	– 0.8400	– 0.6111
Androst-5 -ene-3,17-diol, 3- acetate, (3.β.,17.β.)	+ 1.0000	+ 0.5732	+ 0.5750	+ 0.7143	– 1.0000	– 0.8770	+ 0.8444
γ-Sitosterol	+ 1.0000	+ 0.5385	+ 0.5750	+ 0.5286	– 0.9700	– 0.9000	+ 0.9111
Abieta-8, 11,13-trien- 7-one	+ 1.0000	+ 0.8710	+ 0.7750	+ 0.6000	– 0.8500	– 0.7900	+ 0.5556
Neoabietol	+ 0.9943	+ 0.9493	+ 0.8750	– 0.6143	– 0.8900	– 0.7900	+ 0.6667
Aromadendrene epoxide	+ 0.9913	+ 0.8255	+ 0.9500	+ 0.6429	– 0.7800	– 0.6353	– 0.5000
Neoabietyl acetate	+ 1.0000	+ 0.8454	+ 0.7750	+ 0.5286	– 0.7900	– 0.8900	+ 0.5556
Abitatriene	+ 0.9963	+ 0.9315	+ 0.9500	+ 0.6000	– 0.7000	– 0.8200	– 0.6222

HIA: Human Intestine Absorption; Caco-2: permeability value; BBB: Blood Brain Barrier; HOB: Human Oral Bioavailability.
a: (+): more than 30%, (-): less than 30%.
b: (+): High Caco-2 permeability, (-): moderate-poor Caco-2 permeability.
c: (+) High BBB permeability; (−) moderate-poor BBB permeability.
d: (+) High HOB permeability; (−) moderate-poor HOB permeability.

the safety of the compounds is an important parameter for becoming a good drug (Cheng *et al.*, 2012), Table 7 presents the different values of the pharmacokinetic profile. In this study, no AMES toxicity and carcinogens were identified as a threat. An exception was observed for respiratory toxicity, where some of those compounds posed a positive threat against the respiratory system, such as cedrene, humulene epoxide

II, androst-5-ene-3,17-diol,3-acetate (3.β.,17.β.), abieta-8,11,13-trien-7-one, neoabietol, neoabietyl acetate, and especially γ-sitosterol.

Conclusions

The medicinal plants include natural products such as phytochemicals, used in various medical applications including their antidiabetic effects. Traditionally, *Juniperus phoenicea* berries extracts are considered promising natural medicinal agents and widely used for the treatment of type 2 diabetes (T2D). The findings obtained in this study are divided into three main axes:

- Analysis of the chemical composition of EO, cyclohexane extract, and ethyl acetate extract allowed the identification of50, 60, and 56 compounds, respectively, representing over 98% of the essential oil. Oxygenated monoterpenes are the major components, representing 68% of both non-polar extracts, with oxygenated diterpenes being the major components of the *Juniperus phoenicea* berries from the Aflou region.
- *In vitro* assays for antidiabetic effects, based on the α-amylase inhibitory capability of both non-polar extracts, exhibited a strong inhibitory effect against the α-amylase enzyme in a dose-dependent manner. Maximum inhibition rates of over 75.0% were observed at the high tested dose of 400 mg/mL against α-amylase, with good IC_{50} values for both cyclohexane and ethyl acetate extracts (IC_{50} = 186.91 ± 5.74 mg/mL, IC_{50} = 351.48 ± 0.17 mg/mL) respectively. In comparison, the EO showed a maximum inhibition rate of 33.0% at the high tested dose.
- The study was further enhanced with molecular docking analysis to get a comprehensive understanding of the phenomenon. Various compounds showed promising binding affinity to specific proteins (α-amylase and α-glucosidase), with, all compounds displaying a favorable ADMET profile, indicating their potential to serve as lead compounds in drug discovery. Comprehensive *in silico* studies, including molecular docking studies, were conducted to evaluate and support the *in vitro* findings. However, further studies using different antidiabetic models, especially *in vivo* models, are needed to confirm the beneficial qualities of these extracts.

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Conflicts of interest

The authors declare no conflict of interest.

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