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Essential Oils of *Artemisia herba-alba*, *Mentha pulegium*, and *Cedrus atlantica*: Chemical compositions, in *vitro*, in *vivo*, in *silico* Antifungals Activities, and Genotoxicity

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ABSTRACT

The extraction of three essential oils from medicinal and aromatic plants was carried out using steam distillation. Phytochemical analysis was performed using gas chromatography coupled with mass spectrometry (GC/MS). The results revealed the presence of several bioactive compounds: camphor, identified as the chemotype of the essential oil of Artemisia herba-alba (AhaEO); pulegone, the predominant compound in the essential oil of Mentha pulegium (MpEO); and β-himachalene, the chemotype of the essential oil of Cedrus atlantica (CaEO). The antifungal activity of these essential oils (EOs) was evaluated against Fusarium oxysporum albedinis (Foa) using the direct contact method on PDA medium and in soil. The essential oils exhibited significant antifungal effects. Furthermore, genotoxic effects were assessed, and the results showed that the application of essential oils at concentrations of 2 and 4 µg/mL did not induce DNA damage. Computational simulations and molecular interaction analyses were also performed to validate the experimental findings.

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1. INTRODUCTION

Following the rise of endogenous and aggressive microorganisms that are resistant to synthetic antimicrobials, there is a growing need to decrease the usage of chemicals as antimicrobial agents in the nutrition sector and fight a variety of illnesses [1]. The pursuit of environmentally safe goods has focused on the hunt for basic bioactive chemicals derived from plants that have antifungal properties. The natural mix of monoterpenes, diterpenes, and hydrocarbons found in essential oils is known to have a range of functional groups that give them antifungal and antimicrobial properties [2]. Essential oils of aromatic and medicinal plants cover a wide range of biological activities [3]. Several essential oils have pharmacological benefits, exhibiting anti-inflammatory, antioxidant, and anti-cancerogenic actions.

The genus *Artemisia herba-alba* belongs to asteraceae family [4] is a medicinal and aromatic shrub that grows wild in arid areas of the Mediterranean basin, extending as far as the northwestern Himalayas. The plant is abundant on the Iberian Peninsula, reaching its highest population in central Spain, extending to the east, southeast, and south of Spain [5-6]. Pennyroyal, or *Mentha pulegium*, is a flowering plant that belongs to the Labiatae family. It is native to Europe, North Africa, in Near w East, and Asia Minor [7].

Cedars occur naturally in specific mountainous regions, such as the Mediterranean basin, at altitudes ranging from 1,300 to 2,600 meters above sea level. These include *Cedrus atlantica* Manetti in Morocco and Algeria, *Cedrus brevifolia* Henry in Cyprus, and *Cedrus libani* Loud in Lebanon and Turkey. *Cedrus atlantica*, commonly known as the Atlas cedar, is the most significant species, both in terms of the area it occupies and its annual production. Its populations are divided into seven main blocks, with three located in Algeria and four in Morocco. The Moroccan Atlas block, covering 130,000 hectares, predominantly on calcareous soils from the Lias and Jurassic periods, is the largest of these blocks. It currently accounts for the majority of cedar-sawn timber production [8].

In this study, the antifungal activity of essential oils against Foa was assessed *in vitro, in vivo*, and *in silico*. Additionally, the genotoxic effects of Aha, Mp, and Ca essential oils on rat leukocytes was investigated.

2. METHODS

2.1. Extraction and Characterization of Essential Oils

The treatment in this station goes through several phases shown schematically the various essential oils of artemisia, pennyroyal, and cedar were extracted by steam distillation giving the yield of 1.681, 1.875, and 1.866 % respectively. The chemical composition was analyzed by using GC-MS according to the previously reported method.

2.2. Antifungal Activities

2.2.1. Method of Direct contact on PDA medium (*In vitro*)

The Potato Dextrose Agar (PDA) is used to cultivate Foa. The medium is made by weighing 39 g of PDA, dissolving it in 1L of distilled water, and then boiling the liquid for 10 minutes. After that, it is sterilized for 20 minutes at 120°C.

To prepare the essential oil solution 1 mL of the essential oil is dissolved in 1 mL of dimethyl sulfoxide (DMSO).

Different volumes (50, 100, 150, 200, and 500 µL) of this stock solution are obtained and put in sterile tubes. Sterile liquid PDA is then added to each tube, making the total volume to 15 mL. Poured into petri dishes with a diameter of 8.5 cm, the mixture is left to solidify [9].

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Foa previously grown on solid PDA is then transferred to the center of each petri dish, and the plates are incubated at 28°C for 5 days. The results are expressed as a percentage of inhibition by comparing the diameter of FOA growth in the treated dishes to a control dish containing only PDA, Foa, and 500 µL of DMSO [10].

2.2.2. Effect of essential oils on the development of *Fusaruim Oxysporum albedenis* in soil (*In vivo*)

After sieving the soil with a 1 mm sieve, it was distributed into 100 mL glass vials (Duran vials) at a rate of 50 g of soil per vial and autoclaved for 30 minutes at 120°C and 1 bar.

The autoclaved soil was then inoculated with *Fusarium oxysporum albedinis* at a concentration of 10^4 CFU/g of dry soil, to which the essential oil to be tested was added. The volume of essential oil used corresponded to the amount that achieved total inhibition of *Fusarium oxysporum albedinis* in the in-vitro test (**Table 1**). For the control, the essential oil was replaced with sterile distilled water. Three replicates were performed for each test.

To assess the effect of the tested essential oils on the development of *Fusarium oxysporum albedinis* in soil, 1 g of soil was collected from each vial under sterile conditions and placed into a tube containing 9 mL of sterile distilled water. After shaking for 5 minutes, Petri dishes containing PDA medium were inoculated.

The enumeration of Foa was performed using the suspension dilution method, followed by inoculation on PDA medium. The results, expressed as colony-forming units per gram of dry matter (CFU/g of dry matter), represent the average of three replicates.

Table 1. The volumes of essential oils.

Essential oil	AhaEO	MpEO	CaEO
The added volume of the EO/DMSO mixture (v/v) in µL/g of soil.	10	32	32

2.3. Genotoxicity (Alkaline comet assay)

The essential oils, dissolved in DMSO (dimethylsulfoxide) and PBS, were added to fresh blood from a male Wistar rat to achieve the concentrations (2 and 4 µg/mL), and the cells were treated for 1 h under 37°C. The final DMSO concentration in the media never exceeded 0.2%, and the negative control was exposed to an equivalent concentration of solvent. Alloxan (13.4 mmol/l) was used as a positive control for genotoxic.

10 µL of treated blood cells was dissolved in 200 µl of LMP agarose (low melting point.) (0.5% w/v in a solution of PBS at a temperature of 37°C), the mixture was placed on a slide and quickly covered by the coverslip to form the second layer of agarose. The alkaline comet assay was performed. Comets were visualized using the silver staining method [11]. Slides stained with silver nitrate are viewed under the microscope using the 200x objective. Images are captured using a camera (CMEX 5000). 20 cells (10 cells from each of two replicate slides of each treatment) were selected and analyzed for DNA migration by CaspLab software.

2.4. Computational Simulation and Molecular Interaction Study (*In silico*)

2.4.1. Ligands preparation

To optimize and minimize the energy of ligands identified in the essential oils of Artemisia herba-alba, Mentha pulegium, and Cedrus atlantica using gas chromatography-mass spectrometry (GC-MS), data were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The LigPrep module within Maestro, version 13.8, from Schrödinger LLC (New York, NY, USA) [12], was employed for the preparation of ligands. In

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this study, the antifungal inhibitor Voriconazole was utilized as the standard. The accurate bond order was maintained during the structure preparation with the assistance of the LigPrep package from the Schrödinger software suite. Subsequently, all compounds were converted to SDF format using Maestro and their energy minimized using the OPLS 2005 force field with default parameters [13].

2.4.2. Molecular docking and protein synthesis

Molecular docking investigations were carried out using X-ray crystal structures sourced from the Protein Data Bank. The analyzed structure included the structure of the cytochrome P450 sterol 14-Demethylase (CYP51B) voriconazole complex (PDB ID 4UYM, 2.55 Å resolution) [14]. The preparation of this protein structure was accomplished using the Protein Preparation Wizard in Maestro 13.8 by Schrödinger, LLC (New York, NY, USA). This method involved the removal of ligands and water molecules before merging non-polar hydrogens. The docking target was identified as the active site. A central grid box encompassing all ligand atoms was constructed, with 20 points assigned to each of the three axes (x, y, and z) to ensure an optimal docking environment. Using default settings and an RMSD restriction of 0.3 Å, energy minimization was performed. Binding predictions were generated by selecting relevant poses with the standard precision glide score, and docking scores were presented as binding affinities in kcal/mol. Protein structures were further refined using the OPLS_2005 force field, and the interactions between proteins and ligands were visualized using Biovia Discovery Studio 2021 [15].

2.4.3. ADMET profile of the phytoconstituents

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of compounds from the essential oils of Artemisia herba-alba, Mentha pulegium, and Cedrus atlantica were determined using the QikProp module of the Schrödinger software [16]. Output PDB files generated from this analysis can be examined and verified using appropriate applications. This study revealed important physicochemical properties of the compounds, including their flexibility, molecular weight/size, hydrophobicity, bioavailability, permeability, and polar solubility. Additionally, the assessment applied Lipinski's Rule of Five to evaluate the drug-likeness of the top compounds under investigation, indicating their potential for further development [17].

3. RESULTS AND DISCUSSION

3.1. Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical composition of essential oils from the various studied plants was determined using gas chromatography-mass spectrometry (GC-MS). The results of the analyses are presented in the tables below.

3.1.1. *Artimesia herba alaba*

13 components were detected,with the main being camphor (58.39%), 1,8-cineole (16.25%), camphene (10.76%), thujone (4.17%), and beta-cymene (1.20%) (**Table 2**). The chemotype of *artimesia herba alaba* is camphor. These and other compounds are frequently found in other Moroccan and Mediterranean *artemisia herba alba* essential oils, but at different concentrations depending on geographical origin, phenological stage, environmental factors, extraction methods, and genetic differences [5]. A study shows that the main components of the essential oil of *artemisia herba alba* from the northern Sahara Desert were camphor (49.3%), 1,8-cineole (13.4%), borneol (7.3%), pinocarvone (5.6%),

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camphene (4.9%) and chrysanthenone (3.2%) [18]. Another study on the essential oil of *artemisia herba alba* from the Azzemour region, southwest Morocco, shows that cis-thujone (25.5%), trans-thujone (17.7%), vanillyl alcohol (11.5%), nor-davanone (7.8%) and camphor (4.9%) are the main compounds in the essential oil. Essential oil from the Azrou region in the central Middle Atlas of Morocco contains chrysanthenone (56.8%) as the dominant compound, followed by trans-thujone (31.1%), camphor (0.2%), artemisyl acetate (0.5%) and cis-thujone (0.2%) [19].

Table 2. Chemical composition of *Artemisia herba-alba* essential oil

No	Name	RT (min)	Area (%)
1.	Tricyclene	4.831	0.73
2.	Alpha-pinene	5.003	0.47
3.	Camphene	5.243	10.76
4.	Beta-pinene	5.680	0.41
5.	Yomogi alcohol	5.953	2.56
6.	Beta-cymene	6.400	1.20
7.	D-Limonene	6.468	1.32
8.	1,8-Cineole	6.527	16.25
9.	Ocimene	6.933	0.44
10.	Artemesia alcohol	7.290	2.07
11.	Thujone	7.695	4.17
12.	L-pinocarveol	8.229	1.23
13.	Camphor	8.325	58.39

3.1.2. *Mentha pulegium*

10 components were detected, the main compounds being Pulegone (74.88%), D-Limonene (8.69%), 2-(2,2,4-Trimethyl-3-cyclopenten-1-yl)ethanol (5.43%), verbenone (3.21%) and alpha-humulene (2.18%) (**Table 3**). The chemotype of *mentha pulegium* studied is pulegone. The same main compound, pulegone with different percentages, and other compounds have been found in *mentha pulegium* essential oils from other Moroccan regions. In a study evaluated by Aljaiyash, it was suggested that *mentha pulegium* essential oils from the Marrakech region showed that the main components were pulegone (57.8-62.8%), menthone (9.5-15.0%), limonene (4.9-6.9%). Another study shows that *mentha pulegium* essential oil from the Rabat region contained pulegone (73.33%), menthone (8.63%), and α-pinene (1.70%), as the main compounds [20].

Table 3. Chemical composition of *mentha pulegium* essential oil

No	Name	RT (min)	Area (%)
1.	Alpha-pinene	4.975	1.07
2.	Beta-pinene	5.652	0.79
3.	D-Limonene	6.441	8.69
4.	2-(2,2,4-Trimethyl-3-cyclopenten-1-yl)ethanol	8.300	5.43
5.	Menthone	8.404	0.94
6.	Isomenthol	8.563	1.26
7.	Pulegone	9.682	74.88
8.	Verbenone	11.137	3.21
9.	Caryophyllene	12.213	1.55
10.	Alpha-humulene	12.655	2.18

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3.1.3. *Cedrus atlantica*

17 components were detected (**Table 4**), with the main components being beta-himachalene (49.96%) and alpha-himachalene (19.67%). The same main compound, beta-himachalene, and other compounds were found in *cedrus atlantica* essential oil from other Moroccan regions with different percentages. *cedrus atlantica* essential oil from the Tichoukt region (15 km southeast of Boulmane, Morocco), contained alpha-pinene (14, 85%) followed by himachalene (10.14%), beta-himachalene (9.89%), σ-himachalene (7.62%), cis-alpha-atlantone (6.78%), himachalol (5.26%) and alpha-himachalene (4.15%) as the majority compounds. The main constituents of the essential oil of *cedrus atlantica* specimens collected in the Azrou region, located in the Middle Atlas (Morocco) were cedranone (19, 35%), iso-cedranol (13. 78%), caryophyllene oxide (8.73%), gammacalamenene (7.77%), delta-cadinene (7.34%), beta-himachalene (7.23%), and gammahimachalene (4.05%).

Table 4. Chemical composition of *cedrus atlantica* essential oil.

No	Name	RT (min)	Area (%)
1	1-(4-Methyl-3-cyclohexen-1-yl)ethanone	8.050	0.97
2	Neoisolongifolene, 8,9-dehydro-	11.742	1.17
3	Beta.-Panasinsene	11.967	0.90
4	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]	12.075	0.72
5	Alpha-himachalene	12.625	19.67
6	Beta.-Vatirenene	12.750	0.55
7	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9 tetramethyl(1H)benzocycloheptene	12.975	10.55
8	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methyl ethyl)	13.017	0.90
9	Beta-himachalene	13.258	49.96
10	Isolongifolene, 4,5,9,10-dehydro	13.392	1.85
11	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-, (1S-cis)	13.475	2.95
12	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl	13.642	0.63
13	Alpha.-calacorene	13.750	0.90
14	Epiglobulol	15.208	0.98
15	Tumerone	15.433	0.92
16	3-Hexen-1-ol, 2,5-dimethyl-, formate,(Z)	15.550	1.18
17	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	16.342	4.99

3.2. Antifungal activity

3.2.1. Method of Direct contact on PDA medium (*In vitro*)

The results obtained from the study of the effect of AhaEO, MpEO, and CaEO on the growth of *Fusarium oxysporum albidinis* after a 5-day incubation period at 28°C using the direct contact technique are shown in **Table 5**.

The antifungal activity of essential oils is attributed to the presence of monoterpenic and sesquiterpenic components [21], as well as the synergy between their various compounds. In addition to the diverse biological activities of monoterpenes, essential oils rich in oxygenated monoterpenes have been recognized as important antifungal agents.

3.2.2. Effect of essential oils on the development of *Fusaruim oxysporum albedenis* in soil (*In vivo)*

The results show that the Foa population proliferated significantly in the control, rising from 10^4 CFU/g soil to $3{,}10^4$ CFU/g soil after 5 days, then to $30{,}10^4$ CFU/g soil after 2 months.

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Even after 5 months, it remains very high. However, in the presence of essential oils, the Foa population disappeared completely and immediately. Within 5 days, the Foa population went from 104 CFU/g of soil to 0 colonies. These results remained stable throughout the 5-month experiment (**Figure 1**).

These results reveal that essential oils extracted from aromatic plants (rosemary, Artemisia, and cedar) have a powerful inhibitory effect on the development of Foa in the soil. This may be due to the natural antifungal properties of these essential oils. It would be interesting to pursue this research to understand the precise mechanisms by which these essential oils inhibit Foa growth. This could pave the way for new biological control strategies against this soil-borne pathogen.

Table 5. %inhibition of essential oils.

EO	Volumes (µL)	Diameter (DX) (cm)	% inhibition= (D°-Dx)/D° X 100
	10	4.2	50.59
	20	3.5	58.82
Aha	40	1.8	78.82
	150	0.0	100.00
	200	0.0	100.00
	500	0.0	100.00
	500	0.0	100.00
	10	4.0	52.94
	20	3.3	61.18
	40	0.8	90.59
	50	0.0	100.00
Mp	100	0.0	100.00
	150	0.0	100.00
	200	0.0	100.00
	500	0.0	100.00
	10	6.5	23.53
	20	5.8	31.76
Ca	40	4.7	44.71
	50	4.3	49.41
	100	4.0	52.94
	150	2.3	72.94
	200	2.0	76.47
	500	0.0	100.00

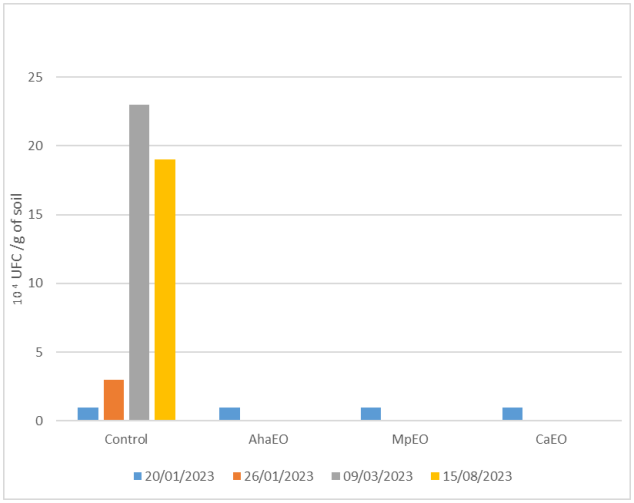

Figure 1. Effect of EOs on the development of foa in the soil.

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3.3. Genotoxicity (Alkaline comet assay)

Genotoxicity refers to the ability of an agent to damage DNA and cause genetic mutations, which can have health implications, particularly in the development of cancer. When a genotoxic agent comes into contact with an organism's cells, it can induce alterations in the DNA sequence, DNA strand breaks, or other genetic damage. These damages can lead to mutations that may have various effects, such as the development of cancers or other genetic diseases. For this reason, it is necessary to test the genotoxicity of natural products before pharmaceutical use.

Our study showed that applying the AhaEo, MpEo and CaEO at concentrations of 2 and 4 µg/mL did not result in DNA damage (**Figure 2**). This was evident by the absence of significant changes in tail length, percentage of DNA in the tail, and tail timing of comets, compared with the negative control. Based on these results, the essential oils tested can be considered non-genotoxic in the concentrations tested. It is important to emphasize that genotoxicity tests are an initial assessment of the safety profile of pharmaceutical products, substances, or nutraceuticals.

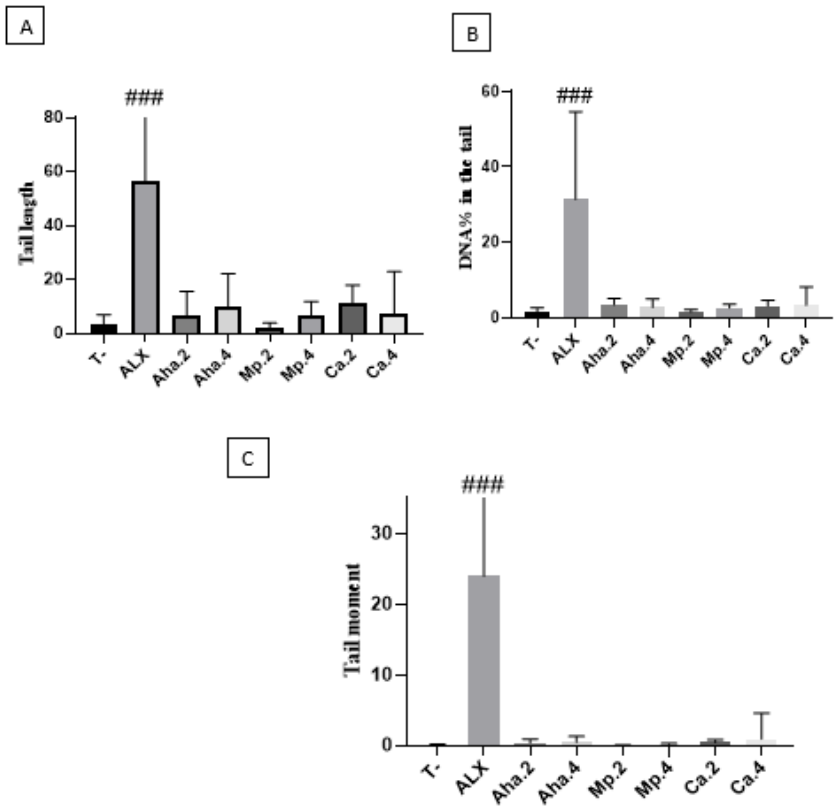

Figure 2. Evaluation of the effect of different concentrations of various essential oils on (A) tail length, (B) percentage of DNA in the tail, and (C) tail moment in rat leukocytes.

Values are expressed as mean ± SEM.
###p < 0.001 compared to the TT- group: Negative control (DMSO-PBS).
ALX: Positive control (Alloxan at 13.4 mmol/L).
Aha.2: AhaEO at a concentration of 2 µg/mL.
Aha.4: AhaEO at a concentration of 4 µg/mL.

Limonene is a compound present in the essential oils studied in the following percentages: AhaEO (1.32%), MpEO (8.69%). Merve Bacanlı *et al*. [22] reported that limonene at concentrations below 10,000 µM did not exert genotoxic effects on lymphocytes.

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The percentage of tail DNA was used to measure DNA damage, and also according to literature data is the most appropriate parameter [23].

In summary, the aggregated data from the publications included in this article, and other publications concerning the comet assay, indicate that the comet assay is a reliable method for detecting DNA damage in the tissues of laboratory animals [24].

3.4. Molecular Docking studies (*In Silico*)

Molecular docking, a crucial computational technique, predicts and analyzes ligand-receptor interactions, including hydrogen bonds, van der Waals forces, and hydrophobic interactions. This method is essential in drug design, offering atomic-level insights into molecular interactions. By simulating ligand-receptor binding, it predicts stable conformations and estimates binding affinities, aiding in identifying potential drug candidates and understanding drug mechanisms. Additionally, it facilitates the design of molecules with enhanced affinity and specificity for their biological targets [25]. All molecules identified in AhaEO, MpEO, and CaEO demonstrated significant inhibitory activity against *Fusarium oxysporum albedinis* (Foa) when compared to the standard, Voriconazole. It was observed that these compounds remained stable in the active site, exhibiting high energy values, with the exception of Yomogi alcohol and Ocimene. The presence of negative and low docking score values indicates that the compounds engaged in strong and favorable binding interactions (**Table 6**). Camphor, identified as the chemotype of the AhaEO, exhibits significant stability within the protein's active site, with a docking score of -5.262 kcal/mol. This compound forms various interactions, including classical hydrogen bonds with LYS271, Pi-alkyl interactions with ALA158 and TRP279, alkyl interactions with ALA158, and van der Waals forces with amino acid residues ASP157, THR155, TYR152, GLY153, and SER274 (**Figure 3**). Pulegone, the predominant compound in the MpEO, also shows high stability within the target protein's active site, with a docking score of -5.543 kcal/mol, comparable to the docking score of voriconazole at -5.584 kcal/mol. This compound forms various interactions within the active site, including Pi-alkyl interactions with CYS463, alkyl interactions with ALA469, PHE456, LEU367, PRO372, ILE373, and ALA307, and van der Waals forces with amino acid residues PHE504, PRO455, ALA371, THR315, SER312, LEU473, and SER311. β-Himachalene, the chemotype of the CaEO, exhibits the highest stability within the target protein's active site, with a docking score of -5.791 kcal/mol, higher than that of voriconazole at -5.584 kcal/mol. This compound forms various interactions within the active site, including Pi-alkyl interactions with CYS463, ILE373, and ALA307, alkyl interactions with ILE373, ILE376, PHE456, CYS463, and ALA307, and van der Waals forces with amino acid residues PRO372, SER311, PHE504, GLY457, and PRO455. These findings highlight the potential efficacy of these natural compounds as antifungal inhibitors against *Fusarium Oxysporum Albidinis*. The essential oils have demonstrated significant antifungal effects, consistent with both *in vivo* and *in vitro* studies. These results underscore the importance of non-covalent interactions in the stability and specificity of the binding.

Table 6. The docking scores of the selected docked compounds identified in AhaEO, MpEO, and CaEO with the protein (4UYM) using SP docking**.**

No	Compound Name	Docking score
1	Alpha-pinene	-5.397
2	Beta-pinene	-5.516
3	1,8-Cineole	-4.575
4	Camphor	-5.262

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Table 7 (continue). The docking scores of the selected docked compounds identified in AhaEO, MpEO, and CaEO with the protein (4UYM) using SP docking.

No	Compound Name	Docking score
5	Caryophyllene	-3.203
6	Camphene	-4.172
7	Thujone	-4.634
8	Yomogi alcohol	-2.960
9	Artemesia alcohol	-5.233
10	Pulegone	-5.543
11	Limonene	-4.318
12	Verbenone	-4.516
13	2-(2,2,4-Trimethyl-3-cyclopenten-1-yl)ethanol	-4.076
14	Alpha-humulene	-5.429
15	Beta-himachalene	-5.791
16	Alpha-himachalene	-4.339
17	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9 tetramethyl(1H)benzocycloheptene	-5.308
18	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	-5.704
19	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)	-4.318
20	Isolongifolene, 4,5,9,10-dehydro	-3.175
21	Menthone	-4.329
22	Isomenthol	-4.694
23	1-(4-Methyl-3-cyclohexen-1-yl)ethanone	-5.493
24	Neoisolongifolene, 8,9-dehydro-	-3.175
25	Beta-panasinsene	-4.314
26	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]	-2.597
27	Beta-vatirenene	-5.101
28	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	-5.803
29	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl	-5.157
30	Alpha-calacorene	-6.362
31	Epiglobulol	-5.587
32	Tumerone	-5.224
33	3-Hexen-1-ol, 2,5-dimethyl-, formate,(Z)	-2.585
34	Tricyclene	-4.382
35	Beta-cymene	-5.563
36	Ocimene	-2.817
37	L-pinocarveol	-5.014
38	Voriconazole	-5.584

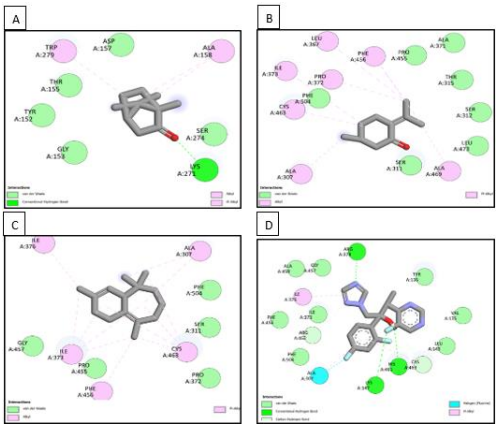

Figure 2. 2D Intermolecular Interactions between (A) Camphor, (B) Pulegone, (C) β-Himachalene, and (D) Voriconazole (standard) with the Active Site of Foa Protein (PDB: 4UYM).

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3.5. *In Silico* Admet Predictions of Pharmacokinetic Studies

Evaluating the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties is crucial in drug development as it determines the pharmacokinetics and safety of candidate compounds [26]. The compounds Camphor, Pulegone, and Beta-himachalene, derived respectively from the AhaEO, MpEO, and CaEO show promising ADMET properties as antifungal inhibitors against *Fusarium oxysporum albidinis* **Tables 7** and **8**. Camphor exhibits good solubility and high cellular permeability (QPPCaco of 4128.173 nm/sec), adhering to all of Lipinski's rules, making it a suitable drug candidate. Pulegone, with even higher permeability (4701.626 nm/sec) and high oral absorption, also shows strong potential as an antifungal inhibitor. Although Beta-himachalene presents a violation of Lipinski's rules (QPlogPo/w > 5), it demonstrates excellent cellular permeability and brain penetration (QPlogBB of 1.068), making it an interesting candidate for optimization. Overall, compounds from the EOs of the three plants show significant antifungal activity, supported by robust interactions with the active sites of target proteins. Compounds such as Alpha-pinene, Beta-pinene, 1,8-Cineole, and Caryophyllene exhibit favorable ADMET properties and good solubility, enhancing their potential as antifungal agents. For example, the high hydrophobicity and cellular permeability observed in these compounds suggest their ability to effectively penetrate fungal cell membranes and disrupt their function. The favorable ADMET properties of these compounds, combined with their strong interactions with the protein active site, confirm their potential as effective antifungal agents. These results, consistent with molecular docking simulations, highlight the importance of non-covalent interactions in the design of new antifungal drugs. The richness of natural compounds, makes Artemisia uses more attractive in medicine as well as other applications as cosmetics and anticorrosion [27-30].

Table 8. *In silico* analysis of lipinski's rule of five for compounds identified in AhaEO, MpEO, and CaEO.

Compound Name	Molecular Weight	Donor HB	Acceptor HB	QPlogPo/w	Rule of five
Alpha-pinene	136.236	0.000	0.000	3.613	0
Beta-pinene	136.236	0.000	0.000	3.505	0
1,8-Cineole	154.25	0.000	0.750	2.435	0
Camphor	152.23	0.000	2.000	1.929	0
Caryophyllene	204.355	0.000	0.000	5.037	1
Camphene	136.23	0.000	0.000	3.310	0
Thujone	152.236	0.000	2.000	2.065	0
Yomogi alcohol	154.252	1.000	0.750	3.008	0
Artemesia alcohol	154.252	1.000	1.700	2.502	0
Pulegone	152.236	0.000	2.000	2.188	0
Limonene	136.236	0.000	0.000	3.981	0
Verbenone	150.220	0.000	2.000	1.894	0
2-(2,2,4-Trimethyl-3-cyclopenten-1-yl)ethanol	154.252	1.000	1.700	2.383	0
Alpha-humulene	204.355	0.000	0.000	5.133	1
Beta-himachalene	204.355	0.000	0.000	5.168	1
Alpha-himachalene	204.355	0.000	0.000	5.184	1
cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9 tetramethyl(1H)benzocycloheptene	204.355	0.000	0.000	5.203	1
1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	206.370	0.000	0.000	5.358	1

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Table 9 (continue). *In silico* analysis of lipinski's rule of five for compounds identified in AhaEO, MpEO, and CaEO.

Compound Name	Molecular Weight	Donor HB	Acceptor HB	QPlogPo/w	Rule of five
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)	204.355	0.000	0.000	5.479	1
Isolongifolene, 4,5,9,10-dehydro	200.323	0.000	0.000	4.811	0
Menthone	154.252	0.000	2.000	2.276	0
Isomenthol	156.267	1.000	1.700	2.736	0
1-(4-Methyl-3-cyclohexen-1-yl)ethanone	138.209	0.000	2.000	1.937	0
Neoisolongifolene, 8,9-dehydro-	200.323	0.000	0.000	4.824	0
Beta-panasinsene	204.355	0.000	0.000	5.092	1
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]	138.209	0.000	2.000	1.932	0
Beta-vatirenene	202.339	0.000	0.000	5.221	1
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	204.355	0	0.000	5.549	1
Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl	204.355	0	0.000	6.258	1
Alpha-calacorene	200.323	0	0.000	5.318	1
Epiglobulol	222.370	1	0.750	4.001	0
Tumerone	218.338	0	2.000	3.687	0
3-Hexen-1-ol, 2,5-dimethyl-, formate,(Z)	165.224	0	2.000	2.231	0
Tricyclene	136.236	0	0.000	4.630	0
Beta-cymene	134.221	0	0.000	3.838	0
Ocimene	136.236	0	0.000	4.403	0
L-pinocarveol	152.236	1	1.700	2.145	0

Rule Of Five*: Number of violations of Lipinski's Rule of Five. The rules are as follows: mol_MW < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. Compounds that satisfy these rules are considered potential drug candidates. (The "five" refers to the limits, which are multiples of 5.)*

Table 10. Predicted *in silico* ADMET analysis for compounds identified in AhaEO, MpEO, and CaEO.

Compound Name	SASA	FOSA	FISA	PISA	PSA	QPPCaco	QPlogBB	HOA
Alpha-pinene	366.0	337.1	0.00	28.9	0.00	9906.038	0.867	3
Beta-pinene	359.7	331.5	0.00	28.2	0.00	9906.038	0.856	3
1,8-Cineole	375.1	375.1	0.00	0.00	7.50	9906.038	0.600	3
Camphor	358.6	318.6	40.0	0.00	24.8	4128.173	0.270	3
Caryophyllene	449.8	419.2	0.00	29.9	0.00	9906.038	1.033	3
Camphene	356.9	330.9	0.00	26.0	0.00	9906.038	0.855	3
Thujone	379.7	332.3	47.4	0.00	28.3	3515.420	0.138	3
Yomogi alcohol	421.8	343.4	420	36.3	21.0	3957.671	-0.022	3
Artemisia alcohol	401.1	333.4	36.4	31.3	19.3	4469.378	0.036	3
Pulegone	392.7	353.0	34.1	5.53	24.3	4701.626	0.229	3
Limonene	386.3	323.6	0.00	62.6	0.00	9906.038	0.840	3
Verbenone	362.9	291.1	52.5	19.3	28.0	3147.204	0.174	3

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Table 11 (continue). Predicted *in silico* ADMET analysis for compounds identified in AhaEO, MpEO, and CaEO**.**

Compound Name	SASA	FOSA	FISA	PISA	PSA	QPPCaco	QPlogBB	HOA
2-(2,2,4-Trimethyl-3-cyclopenten-1-yl)ethanol	403.4	321.5	55.9	25.9	23.0	2921.149	-0.070	3
Alpha-humulene	455.5	425.3	0.00	30.1	0.00	9906.038	1.044	1
Beta-himachalene	457.2	437.5	0.00	20.4	0.00	9906.038	1.068	3
Alpha-himachalene	458.4	424.7	0.00	33.7	0.00	9906.038	1.042	3
cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9 tetramethyl(1H)benzocycloheptene	459.8	433.1	0.00	26.7	0.00	9906.038	1.059	1
1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl	468.0	420.5	0.00	47.5	0.00	9906.038	1.034	1
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)-, (1S-cis)	476.6	463.2	0.00	13.3	0.00	9906.038	1.120	1
Isolongifolene, 4,5,9,10-dehydro	431.8	285.4	0.00	146	0.00	9906.038	0.763	3
Menthone	387.5	349.5	37.9	0.00	0.00	4326.363	0.213	3
Isomenthol	397.6	357.9	39.7	0.00	0.00	4160.266	0.130	3
1-(4-Methyl-3-cyclohexen-1-yl)ethanone	375.4	297.7	42.7	34.9	0.00	3894.128	0.162	3
Neoisolongifolene, 8,9-dehydro-	432.5	286.4	0.00	146	0.00	9906.038	0.766	3
Beta-panasinsene	453.3	429.6	0.00	23.7	0.00	9906.038	1.052	3
1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S(1.alpha.,3a.beta.,4.alpha.,8a.beta.)]	375.8	296.4	43.3	36.1	0.00	3848.273	0.56	3
Beta-vatirenene	457.5	351.3	0.00	106.2	0.00	9906.038	0.895	3
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methyl ethyl)	480.4	459.3	0.00	21.0	0.00	9906.038	1.112	1
Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl	520.9	469.1	0.00	51.7	0.00	9906.038	1.131	1
Alpha.-calacorene	463.2	354.8	0.00	108.4	0.00	9906.038	0.903	3
Epiglobulol	468.3	440.1	28.1	0.00	0.00	5359.948	0.282	3
Tumerone	481.7	384.8	34.6	57.3	0.00	4167.757	-0.001	3

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Table 12 (continue). Predicted *in silico* ADMET analysis for compounds identified in AhaEO, MpEO, and CaEO**.**

Compound Name	SASA	FOSA	FISA	PISA	PSA	QPPCaco	QPlogBB	HOA
3-Hexen-1-ol, 2,5-dimethyl-, formate,(Z)	418.2	322.2	86.6	9.28	0.00	1492.933	-0.387	3
Tricyclene	353.9	353.9	0.00	0.00	0.00	9903.038	0.901	3
Beta-cymene	384.7	254.5	0.00	130.2	0.00	9906.038	0.702	3
Ocimene	411.0	344.7	0.00	66.28	0.00	9906.038	0.882	3
L-pinocarveol	372.4	304.1	40.9	27.40	0.00	4052.534	0.195	3

4. CONCLUSION

The chemical composition of essential oils from aromatic and medicinal plants highlights their rich diversity of bioactive compounds. Notably, Aha, Mp and Ca oils exhibit promising antifungal activities and demonstrate antigenotoxic properties, making them valuable for pharmaceutical and industrial applications. Future research should focus on standardizing methods and exploring their mechanisms to fully harness their potential.

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6. AUTHORS’ NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

7. REFERENCES

[1] Nazzaro, F., Fratianni, F., Coppola, R., and De Feo, V. (2017). Essential oils and antifungal activity. *Pharmaceuticals*, *10*(4), 86.

[2] Sridhar, S. R., Rajagopal, R. V., Rajavel, R., Masilamani, S., and Narasimhan, S. (2003). Antifungal Activity of Some Essential Oils. *Journal of Agricultural and Food Chemistry*, *51*(26), 7596–7599.

[3] Kalemba, D., and Kunicka, A. (2003). Antibacterial and Antifungal Properties of Essential Oils. *Current Medicinal Chemistry*, *10*(10), 813–829.

[4] Rolnik, A., and Olas, B. (2021). The plants of the asteraceae family as agents in the protection of human health. *International Journal of Molecular Sciences*, *22*(6), 3009.

[5] Belhattab, R., Amor, L., Barroso, J. G., Pedro, L. G., and Cristina Figueiredo, A. (2014a). Essential oil from Artemisia herba-alba Asso grown wild in Algeria: Variability assessment and comparison with an updated literature survey. *Arabian Journal of Chemistry*, *7*(2), 243–251.

[6] Ferchichi, A. (1997). Contribution à l’étude cytotaxonomique et biologique d’Artemisia herba-alba Asso en Tunisie présaharienne. *Acta Botanica Gallica*, *144*(1), 145–154.

[7] Mahboubi, M., and Haghi, G. (2008). Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *Journal of Ethnopharmacology*, *119*(2), 325–327.

[8] Zaremski, A., Bakkali-Yakhlef, S., Chaintreuil, C., Abbas, Y., Prin, Y., Abourouh, M., Ducousso, M., and Baudasse, C. (2007). Caractérisation moléculaire du M'jej, agent de dépérissement des cédraies marocaines. *Bois and Forets des tropiques*, *291*, 67–73.

[9] Neri, F., Mari, M., and Brigati, S. (2006). Control of Penicillium expansum by plant volatile compounds. *Plant Pathology*, *55*(1), 100–105.

[10] Hmouni, A., Hajlaoui, M. r., and Mlaiki, A. (1996). Résistance de Botrytis cinerea aux benzimidazoles et aux dicarboximides dans les cultures abritées de tomate en Tunisie. *EPPO Bulletin*, *26*(3–4), 697–705.

[11] Nandhakumar, S., Parasuraman, S., Shanmugam, M. M., Rao, K. R., Chand, P., and Bhat, B. V. (2011). Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay). *Journal of Pharmacology and Pharmacotherapeutics*, *2*(2), 107–111.

[12] Boumezzourh, A., Ouknin, M., Merzouki, M., Dabbous-Wach, A., Hammouti, B., Umoren, P. S., Costa, J., Challioui, A., Umoren, S. A., and Majidi, L. (2023). Acetylcholinesterase, Tyrosinase, α-Glucosidase inhibition by Ammodaucus leucotrichus Coss. and Dur. Fruits Essential oil and Ethanolic Extract and Molecular Docking Analysis. *Moroccan Journal of Chemistry*, *11*(04), 4-11.

[13] Bouakline, H., Bouknana, S., Merzouki, M., Ziani, I., Challioui, A., Bnouham, M., Tahani, A., and Bachiri, A. E. (2024). The Phenolic Content of Pistacia lentiscus Leaf Extract and Its Antioxidant and Antidiabetic Properties. *The Scientific World Journal*, *2024*(1), 1998870.

[14] Titi, A., Touzani, R., Moliterni, A., Hadda, T. B., Messali, M., Benabbes, R., Berredjem, M., Bouzina, A., Al-Zaqri, N., Taleb, M., Zarrouk, A., and Warad, I. (2022). Synthesis, structural, biocomputational modeling and antifungal activity of novel armed pyrazoles. *Journal of Molecular Structure*, *1264*, 133156.

[15] Ouahabi, S., Daoudi, N. E., Loukili, E. H., Asmae, H., Merzouki, M., Bnouham, M., Challioui, A., Hammouti, B., Fauconnier, M.-L., Rhazi, L., Ayerdi Gotor, A., Depeint, F., and Ramdani, M. (2024). Investigation into the Phytochemical Composition, Antioxidant Properties, and In-Vitro Anti-Diabetic Efficacy of Ulva lactuca Extracts. *Marine Drugs*, *22*(6), 240.

[16] Abbaoui, Z., Merzouki, M., Oualdi, I., Bitari, A., Oussaid, A., Challioui, A., Touzani, R., Hammouti, B., and Diño, W. A. (2024). Alzheimer's disease: In silico study of rosemary diterpenes activities. *Current Research in Toxicology*, *6*, 100159.

[17] Bekkouch, A., Merzouki, M., El Mostafi, H., Elhessni, A., Challioui, A., Mesfioui, A., and Touzani, R. (2024). Potential inhibition of ALDH by argan oil compounds, computational approach by docking, ADMET and molecular dynamics. *Moroccan Journal of Chemistry*, *12*(2), 676–695.

[18] Dahmani-Hamzaoui, N., and Baaliouamer, A. (2010). Chemical composition of Algerian Artemisia herba-alba essential oils isolated by microwave and hydrodistillation. *Journal of Essential Oil Research*, *22*(6), 514-517.

[19] Aljaiyash, A., Kasrati, A., Alaoui Jamali, C., and Chaouch, A. (2018). Effect of cultivation on chemical composition and bioactivities of essential oils from *Artemisia herba-alba* Asso grown in Morocco. *Biochemical Systematics and Ecology*, *81*, 74–79.

[20] Benayad, N., Ebrahim, W., Hakiki, A., and Mosaddak, M. (2012). Chemical

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p- ISSN 2776-6098 e- ISSN 2776-5938

characterization and insecticidal evaluation of the essential oil of Mentha suaveolens L. AND Mentha pulegium L. growing in Morocco. *Scientific Study and Research: Chemistry and Chemical Engineering, Biotechnology, Food Industry*, *13*(1), 27–32.

[21] Danielli, L. J., de Souza, T. J., Maciel, A. J., Ferrão, M. F., Fuentefria, A. M., and Apel, M. A. (2019). Influence of monoterpenes in biological activities of Nectandra megapotamica (Spreng.) Mez essential oils. *Biomolecules*, *9*(3), 112.

[22] Bacanlı, M., Başaran, A. A., and Başaran, N. (2015). The antioxidant and antigenotoxic properties of citrus phenolics limonene and naringin. *Food and Chemical Toxicology*, *81*, 160–170.

[23] Edziri, H., Guerrab, M., Anthonissen, R., Mastouri, M., and Verschaeve, L. (2020). Phytochemical screening, antioxidant, anticoagulant and *in vitro* toxic and genotoxic properties of aerial parts extracts of *Fumaria officinalis* L. growing in Tunisia. *South African Journal of Botany*, *130*, 268–273.

[24] Møller, P. (2005). Genotoxicity of environmental agents assessed by the alkaline comet assay. *Basic and Clinical Pharmacology and Toxicology*, *96 Suppl 1*, 1–42.

[25] Wihadi, M. N. K., Merzouki, M., Ma'arif, A. S., Grasianto, G., and Santosa, S. J. (2024). Insights of auric ion adsorption in the presence of ferric and hexavalent chromium species on Mg/Al layered double hydroxides. *Moroccan Journal of Chemistry*, *12*(2), 854–869.

[26] Loukili, E. H., Merzouki, M., Taibi, M., Elbouzidi, A., Hammouti, B., Kumar Yadav, K., Khalid, M., Addi, M., Ramdani, M., Kumar, P., and Ryeol Choi, J. (2024). Phytochemical, biological, and nutritional properties of the prickly pear, *Opuntia dillenii*: A review. *Saudi Pharmaceutical Journal*, *32*(10), 102167.

[27] Bammou, L., Mihit, M., Salghi, R., Bazzi, L., Bouyanzer, A., Al-Deyab, S.S., Hammouti B. (2011), Inhibition effect of natural artemisia oils towards tinplate corrosion in HCl solution: Chemical characterization and electrochemical study. *International Journal of Electrochemical Science,* 6(5), 1454-1467.

[28] Ouachikh O., Bouyanzer A., Bouklah M., Desjobert J-M., Costa J., Hammouti B., Majidi L. (2009), Application of Essential Oil of Artemisia herba alba as green corrosion inhibitor for steel in 0.5M H_2SO_4, *Surface Review and Letters*, 16(1), 49-54.

[29] El Mchichi, L., El Aissouq, A., Kasmi, R., Belhassan, A., El-Mernissi, R. et al. (2021). In silico design of novel Pyrazole derivatives containing thiourea skeleton as anti-cancer agents using: 3D QSAR, Drug-Likeness studies, ADMET prediction and molecular docking, *Materials Today: Proceedings* 45, 7661-7674.

[30] El Ouardi M, Drioiche A, El Makhoukhi F, Mabrouki J, Hakmi M, Al kamaly O, A. Alsfouk B, Eddamsyry B, Khamar H, Zair T and Alaoui El Belghiti M (2024) Chemical composition, antimicrobial, and antioxidant properties of essential oils from Artemisia herba-alba asso. and Artemisia huguetii caball. from Morocco: in vitro and in silico evaluation. *Frontiers in Chemistry,* 12, 1456684.

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