JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

VALORIZATION OF PERILLALDEHYDE MOLECULE CONTAINED IN THE ESSENTIAL OIL OF *Ammodaucus Leucotrichus* Coss. FROM THE SAHARAN ZONES OF MOROCCO

*Omayma Bouzekri**[1], Sabah El Gamouz[1], Adib Ghaleb[2], Ali Amechrouq[1], Mostafa El Idrissi[1], M'barek Choukrad[1]*

Address(es):
[1]Molecular Chemistry and Natural Substances Laboratory, Moulay Ismail University, Faculty of Sciences, B.P. 11201, Zitoune, Meknes, Morocco.
[2]Analytic and Molecular Chemistry "LCAM" laboratory, Cadi Ayyad University, Faculty Polydisciplinary of Safi, Safi 46030 Morocco.

*Corresponding author: omayma.bk.ouma@gmail.com

https://doi.org/10.55251/jmbfs.4324

ARTICLE INFO

Received 10. 2. 2021
Revised 13. 8. 2021
Accepted 2. 9. 2021
Published 1. 2. 2022

Regular article

OPEN ACCESS

ABSTRACT

The aim of this work is to valorise the species *Ammodaucus leucotrichus* Coss which is known by its abundance in perillaldehyde. The plant material was collected from two regions in Morocco: Errachidia and Smara region, then was hydrodistilled. The essential oils (EOs) obtained were firstly characterized by gas chromatography coupled to mass spectrometry (GC-MS). Secondly the EO with the major component is the perillaldehyde was used as starting material to semi-synthesize three *p*-menthane monoterpenoids. The semi-synthesized compounds from perillaldehyde were studied for their docking behavior against Lung Cancer with the crystallographic structure of the kinase domain of EGFR protein using surflex-docking.
The GC/MS analysis of essential oils, showed a total of 25 components accounts 99.51% for Errachidia's EO while 28 components account for 99.83% for Smara's EO. GC/MS analysis showed that perillaldehyde is the major components in the EO of Errachidia with a percentage of 74.71%. For this purpose, Errachidia's EO was used to semi-synthesize the perilla alcohol, the perillic acid, and the perillartine. The three compounds were obtained in good yield, their structures were confirmed by GC/MS and Infrared (IR). The molecular docking study showed a good binding affinity between the perilla alcohol (POH) and the lung cancer receptor than perillic acid and perillartine.
The species *Ammodaucus leucotrichus Coss*. could be considered a promising source of *p*-menthan monoterpenoids, known for their anticancerous properties.

Keywords: *Ammaudocus leucotrichus Coss.*; Essential oil; molecular docking; perillaldehyde; Semisynthesis

INTRODUCTION

Lung cancer is the most fatal type of cancer. The percentage of deaths because of this cancer account for about 19 % of all cancer deaths **(Torre et al., 2016)**. Lung cancer is the leading cause of cancer death in men and the second leading cause of cancer death in women worldwide **(Torre *et al*., 2016).** Recently, medical research has given notable attention to natural products in the field of cancer treatment **(Prakash *et al*., 2013)**. More than two-thirds of the drugs currently used in cancer treatments come directly from natural products **(Gurnani et *al*., 2014)**. Much recent researches have great interest in screening of medicinal plants for new therapeutics (**Bhuyar *et al.* 2021)**. Because of their efficacy, safety, and availability **(Naczk *et al*., 2006)**. The secondary metabolites existed in plants are manly used as active principals of many drags (**Bhuyar *et al.* 2021)**. *Ammodaucus leucotrichus* Coss belongs to the apiaceae family and Ammodaucus genus. This species is widespread in Saharan and sub-Saharan countries, and it is known to be endemic to North Africa, specifically Morocco, Algeria, and Tunisia, extending to Egypt and tropical Africa in addition to the Canary Islands **(Manssouri *et al*., 2016)**. Previous studies on the volatile compounds of *Ammodaucus leucotrichus* Coss. esseantial oil, have revealed the existence of perillaldehyde among its chemical composition. **(Abu Zarga *et al*., 2013; El-Haci *et al*., 2014; Manssouri *et al*., 2020)**. perillaldehyde is a monoterpenoid with p-menthane structure. Monoterpenoid compounds with p-menthane are known for their antioxidant activity and anticancer potential. Moreover, the compounds with this structure are reported to possess in vitro cytotoxic effects on cancer cell lines **(Imamura *et al*., 2014)**. The monoterpene perilla alcohol is the most promising member of the group p-menthanes, this compound has attracted attention recently by being a strong candidate for cancer treatment **(Yeruva *et al*., 2007)**. Perilla alcohol is known for its bioactivity as an antitumor agent and inhibitor of tumor cell growth in various tissues **(Imamura *et al*., 2014)**. Clinical trials were conducted on the toxicity and chemotherapy effects of Perilla alcohol **(Raphael *et al*., 2003; Yeruva *et al*., 2007)**. Another group found that limonene and perillic acid inhibited DNA synthesis and proliferation in PHA-stimulated peripheral blood mononuclear cells and perillic acid inhibited IL-2 and IL-10 production and secretion in mitogen-activated human T lymphocytes **(Swain *et al*., 2020)**. As well, Perillartine is considered a monoterpene with antioxidant and radical scavenging activities **(Swain *et al*., 2020)**.
The objective of this study was a valorization of the species *Ammodaucus Leucotrichus* Coss., to confirm the potential of this species in the pharmaceutical industry as a source of perillaldehyde. For this purpose, plant material was collected in two Saharan regions in Morocco: (Draa-Tafilalet and Laâyoune-Sakia El Hamra). The chemical composition of the essential oils was analyzed by gas chromatography-mass spectrometry (GC/Ms) analyses with the aim of using the major component in the essential oil which is the perillaldehyde to semi-synthesize bioactive compounds of the *p*-menthane structure which are perilla alcohol (POH), perillic acid and perillartine. As these compounds are known for their anticancer activity, a theoretical study of these compounds against lung cancer, was carried out. The molecular docking behavior of perilla alcohol (POH), perillic acid and perillartine with the crystallographic structure of the kinase domain of the EGFR protein was investigated using surflex-docking.

MATERIAL AND METHODS

Molecular Docking

The bioactive compounds Perilla alcohol, Perillic acid and Perillartine are known for their anticancer activity, therefore, the three compounds were used for molecular docking against Lung Cancer. The crystal structure of EGFR kinase domain in complex with Iressa was downloaded from the PDB data bank with PDB entry: 2ITY **(Yun *et al*., 2007)** and was used as the initial 3D model to determine the docking–binding models. Surflex-Dock module is performed for molecular docking; the protocol of receptor pocket was established by the ligand based method.

Protein target preparation

All water molecules in crystal structures were deleted and the polar hydrogen atoms were added. The IRE ligand (native ligand) of the downloaded structure was retrieved and redocked in the same pocket to determine the performance of this molecular docking study. All studied ligands are docked in the same pockets for further analysis applying an automatic docking. Total scores are expressed in − log 10(Kd) units, in order to present the binding affinities **(Yadav *et al*., 2018)**. Surflex-Dock scores represent binding energies; therefore, they determine ligand-receptor interactions.

Ligands preparation

The chemical structure of all ligands was designed using Sybyl-x 2.0 drawing interface. The conformation of every compound is energetically minimized using Tripos force field and Powell conjugated gradient algorithm with a convergence criterion of 0.05 kcal/mol and Gasteiger-Huckel charges **(Purcell *et al*., 1967)**.

Lipinski rule of bioactive compounds Perilla alcohol, Perillic acid and Perillartine

The main goal to verify Lipinski rule is to obtain a preliminary prediction of the potential pharmacological capacities of a molecule to become a drug. The tree studied compounds were submitted to the calculation of their physicochemical properties such as molecular weight (MW), octanol/water partition coefficient (LogP), number of rotatable bond (N_{rot}) hydrogen bond donor (HBD) and acceptor (HBA) using SwissADME webserve **(Daina *et al*., 2017)**.

The plant material

The fruits of *Ammodaucus leucotrichus Coss*. Were collected in April 2018 from two different regions of Morocco: Errachidia (ERR) region: Draa-Tafilalet and Smara (SM) region: Laâyoune-Sakia El Hamra. The geographical coordinates of Errachidia are: 31°07'10 "N -5°09'58 "W and those of Smara are: 26°45'32.1 "N 11°41'10.6 "W.
The fruits were dried in the dark, the extraction of essential oils was carried out using the method of hydrodistillation by Clevenger device. 100 g of fruits was powdered then extracts. The hydrodistillation was repeated three times and the obtained essential oils were dried with anhydrous sodium sulfate and stored at 4°C in amber glass flasks until analysis.

Analysis of essential oil by GC/MS

The constituents of the essential oils were analyzed by the GC-Ms method. GC-Ms analyses were performed using Thermo Scientific ISQ Series Quadrupole GC-MS System. The GC instrument was equipped with a TG-1MS capillary column (30 m × 0.32 mm i.d., 0.25µm film thickness). Here the carrier gas used was Helium with a flow rate of 1.5 ml/min and EI source at 70 eV. The GC oven was held at a temperature of 40°C at the initial time and programmed to 200°C at 4°C/min and up to 300°C at 30°C/min and maintained for 10 min. 1µl of the dilute sample (in cyclohexane 1:100, v/v) was injected with a constant temperature of 250°C through a split injector. The identification of chemical components was carried out by the comparison of the Chemical Abstract Service registry number (Cas) and matching their mass spectra value with the corresponding database NIST library and with mass spectral literature (the Reverse Match Factor RSI > 600) **(Lal *et al*., 2019b, 2019a,)**.

Semi-synthesis of Perilla alcohol, Perillic acid and Perillartine from the perillaldehyde

Reduction of perillaldehyde to Perilla alcohol with $NaBH_4$

In order to reduce carbonyl compounds to their corresponding alcohols, lithium aluminum hydride ($LiAlH_4$) or sodium borohydride ($NaBH_4$) are frequently used **(Setamdideh *et al*., 2012)**. In this case, the $NaBH_4$ was used because it is more selective to carbonyl group. In a Three-neck round-bottom flask equipped with a dropping funnel and with nitrogen sparging, 4 equimolar of the aldehyde were added drop-wise to a mixture of NaBH4 solubilized in diethyl ether and ethanol. The reaction was stirred for 24h in ice. After the reaction was complete, 12 mL of distilled water was added and the mixture was extracted with diethyl ether (3 × 20 mL) and dried over anhydrous magnesium sulfate. The diethyl ether was evaporated in a rotary evaporator.

*Disproportionation of P*erillaldehyde *into Perilla alcohol and Perillic acid*

Perillaldehyde (PA) has no alpha hydrogen therefore, cannot be subjected to aldol condensation, but may be disproportionate to an equimolar mixture of primary alcohol and carboxylic salt. The reaction is managed in a strongly basic solution. A concentrated sodium hydroxide solution was prepared in a 100 mL round-bottom flask adapted with a refrigerant. After dissolution, PA was added to the hot sodium hydroxide solution and agitated until an emulsion was produced. The solution was maintained under reflux during 24 hours. After the reaction was complete, a mixture of POH and perillic acid sodium salt was obtained. The mixture was transferred into a separating funnel and extracted with diethyl ether (3×15 mL). The ethereal phases were collected, dried and evaporated and the aqueous phase was acidified with concentrated HCl to the pH acid, then stirred with cooling a white precipitate appeared.

Preparation of perillaldehyde oxime

The synthesis of the aldoxime was done by using a 100 mL round flask equipped with a refrigerant and dropping funnel. 2.5g of Hydroxylammonium chloride $[NH_3OH]Cl$, 4g of sodium acetate $C_2H_3NaO_2$ and 10 mL of water were placed in the round flask, the mixture was stirred until dissolution, then the solution was heated (40 °C) 2.5 mL of PA was wise dropped, after the appearance of the oxime it was filtered then washed with ice water.

RESULTS AND DISCUSSION

Molecular Docking

The compounds Perilla alcohol (POH), Perillic acid, and Perillartine which are known for their lung cancer inhibition **(Yeruva *et al*., 2007)** were studied for their docking behavior with the crystallographic structure of the kinase domain of EGFR protein retrieved from the Protein Databank repository (www.rcsb.org/pdb; ID: 2ITY) using surflex-docking. The stable conformation of Perilla alcohol (POH) compound shows better binding affinity than Perillic acid and Perillartine with a scoring of 4.18, 3.80 and 3.56 respectively (see Table 1).

Table 1 Summery of affinities and type's interactions between studied compounds and lung cancer receptor

Stable conformation of compound	Score	Hydrogen Interactions with residue(s)	Alkyl Interactions with residue(s)
Perilla alcohol	4.18	PRO A:794	LEU A :718; LEU A :844; ALA A : 743; VAL A:726; LEU A:792
Perillic acid	3.80	MET:793; GLN A:791	LEU A: 718; CYS A: 797; ALA A: 743; LEU A: 844
Perillartine	3.56	Lys A: 745	VAL A:726; LEU A:718; LEU A: 792; LEU A : 844 ; ALA A : 743; MET : 793

Hydrogen bonding is one of the significant factors to determine the stability of protein-ligand bonding. The compound Perilla alcohol (POH) is stabilized by hydrogen bond interaction between hydroxyl group and PRO A:794 residue, and by alkyl interactions with LEU A:718; LEU A:844; ALA A:743; VAL A:726; LEU A:792 residues. The green and brown contours around hydroxyl group indicate the hydrophobicity and hydrogen bond characters of receptor that stabilize Ligand-receptor complex, therefore the inhibition ability (see Figure 1). The Perillic acid forms two hydrogen bonds with MET :793 and GLN A:791 residues, also alkyl interactions with LEU A: 718; CYS A: 797; ALA A: 743 and LEU A: 844 residues (see Figure 2). The Perillartine forms a hydrogen bond with Lys A: 745 residue and alkyl interactions with VAL A: 726; LEU A:718; LEU A: 792; LEU A: 844; ALA A: 743; MET: 793 residues (see Figure 3). The brown color around the compounds shows the hydrophobic character of amino acids and the green color indicate the important of hydrogen bond interaction to stabilize the affinity of the complex.

Drug-likeness Lipinski's rule

Depending on lipinski's rule compounds with more than 5 hydrogen bond donor, 10 hydrogen bond acceptor, molecular weight (MW) over than 500 Da and LogP more than 5, are poor absorbed drugs **(Lipinski *et al*., 2001)**. The in silico results of the three bioactive compounds, indicate that all compounds have LogP value under 5, and their molecular weight less than 500 Da with hydrogen bond donor and acceptor less than five with two rotatable bonds (see Table 2), which is in agreement with lipinski's rule therefore indicate a good oral bioavailability.

Table 2 Physicochemical parameters of the three synthesized compounds

Compound	LogP	MW	HBD	HBA	N_{rotb}
Perilla alcohol	2.5	152.23	1	1	2
Perillic acid	2	166.22	1	2	2
Perillartine	2.32	165.23	1	2	2

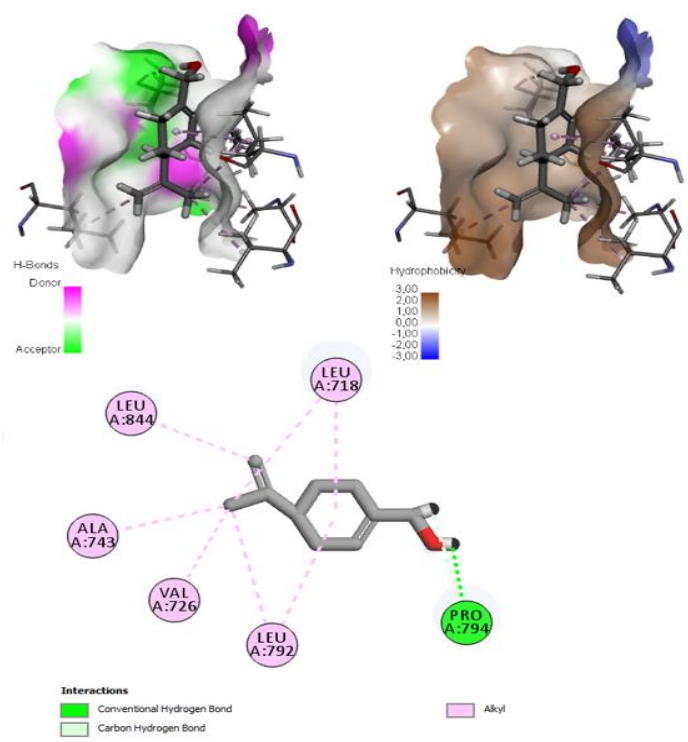


Figure 1 The interactions between compound Perilla alcohol (POH) and PDB:2ITY receptor

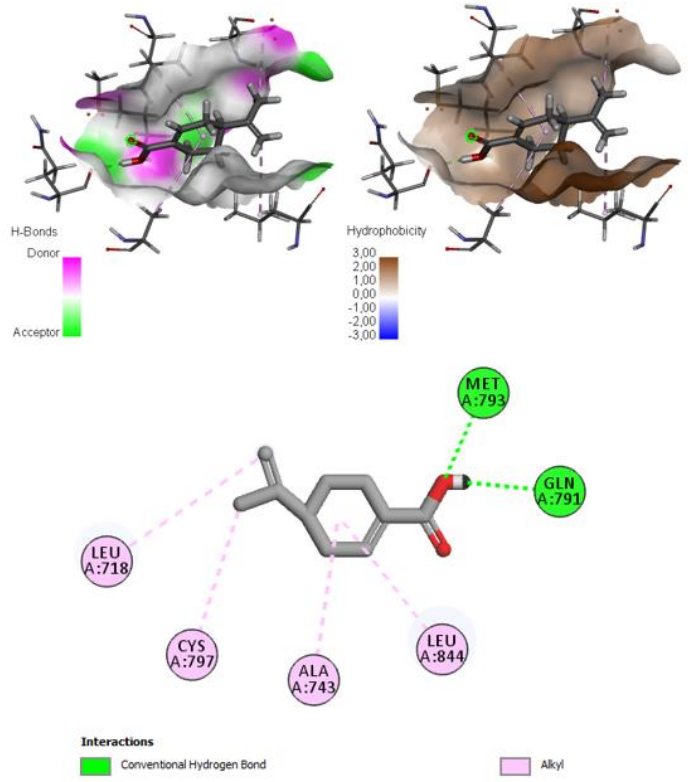


Figure 2 The interactions between compound Perillic acid and PDB:2ITY receptor

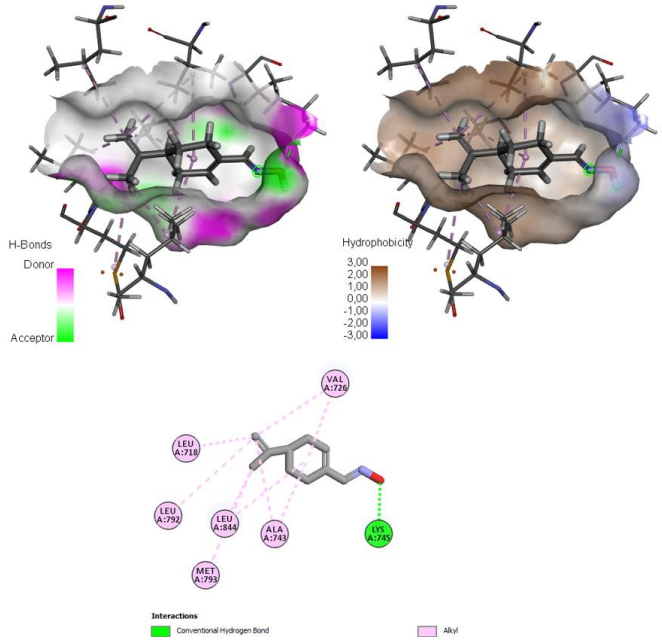


Figure 3 The interactions between compound Perillartine and PDB:2ITY receptor

Analysis of essential oil by GC/MS

The hydro-distillation of *Ammodaucus leucotrichus* fruits from both ERR and SMR regions yielded a liquid blue oil with a characteristic odor. The EO of *Ammodaucus leucotrichus* fruits from ERR region recorded the highest yield of 4.26g; on the other hand, the fruits from SMR region recorded a yield of 3.87g.
The results of GC-Ms analysis of the two essential oils are presented in Table 3.The Retention Indices indicated in the table was determined based on the GC/Ms and literature surveys **(Babushok *et al*., 2011).** The EO from ERR contains a total of 25 components and accounts for 99.51%, essentially the oxygenated monoterpenoids and the monoterpenoids hydrocarbons, whereas the total identified components of the EO from SMR was 28 components which accounts for 99.83%. Similarly, to the EO from ERR, mainly the monoterpenoids were identified; the percentage of the monoterpenoids hydrocarbons was higher followed by the oxygenated monoterpenoids. In both oils the sesquiterpenoids and the oxygenated monoterpenoids were identified but in lesser amounts. The major components were Perillaldehyde, D-limonene, α-Pinene, β-Pinene, Methyl perillate, 3-Carene, β-Myrcene and Perilla alcohol. Comparing their percentages, although the percentage of perillaldehyde and Perilla alcohol in the EO of ERR was higher than the EO of SMR, the percentages of α-Pinene, β-Pinene, Methyl perillate, 3-Carene and β-Myrcene, were higher in the EO of SMR. There are 8 components in the EO of ERR that were not present in the EO of SMR which account for 0.49%. There were 10 components with a percentage of 0.46% missing in the EO of ERR, and 17 components were identified in both EOs. Different factors may be responsible for this quantitative difference including the difference in environmental factors **(Gogoi *et al*., 2018)** the climate, the soil nature, and the period of vegetative cycle **(Noudjou *et al*., 2007)**.

Semi-synthesis of Perilla alcohol, Perillic acid and Perillartine from the Perillaldehyde

According to the chemical components of *Ammodaucus leucotrichus* essential oil from the two regions, the percentage of perillaldehyde in the
EO from ERR was 74.71%. This high percentage was exploited to hemi-synthesis another bioactive component.
The reduction of perillaldehyde with $NaBH_4$ gave an oily liquid, with a green color and characteristic odor after the evaporation of the diethyl ether. With a yield of 97.64%, the essential oil was analyzed by IR before and after reduction (see figure 4) according to the spectrum a total disappearance of the CO band of carbonyl was observed around 1678 cm^{-1} and the appearance of the OH band of alcohol around 3396 cm^{-1}. The obtained EO was also analyzed by GC-Ms. The comparison of the mass spectra obtained for the sample and those of the built-in libraries NIST, showed the match of the samples chromatogram with Perilla alcohol chromatogram (a) (see figure 5).

Table 3 GC–MS analysis of Chemical composition of *Ammodaucus leucotrichus* Coss. & Dur. essential oils.

Sr No.	Components	RT	RI(DIMS)	Percentage area of the EO from ERR %	Percentage area EO from SMR %
1	α-Pinene	7.83	934.5	1.68	2.40
2	Camphene	8.21	947.4	0.14	0.20
3	β-Pinene	9.15	973.1	0.78	2.20
4	β-Myrcene	9.87	983.1	0.30	0.56
5	α-Phellandrene	10.18	999.1	0.10	0.20
6	3-Carene	10.46	1007.2	1.01	2.16
7	o-Cymene	10.76	1032	0.06	0.04
8	D-Limonene	11.16	1023.7	17.57	29.78
9	Terpinolene	13.19	1079.3	___	0.05
10	trans-p-Mentha-2,8-dienol	14.06	1107.0	0.05	___
11	cis- Limonene oxide	14.48	1117.9	0.02	___
12	trans- Limonene oxide,	14.64	1122.7	0.04	___
13	trans-Verbenol	14.93	1133.7	0.03	0.03
14	Verbenone	16.68	1184.4	___	0.02
15	Isopiperitenol B	16.76	___	0.03	___
16	L-Perillaldehyde	17.34	___	0.21	___
17	Cumic aldehyde	17.79	1212.6	0.22	0.12
18	3-Caren-10-al	18.56	1170.8	0.07	___
19	Myrtenal	18.57	1170.8	___	0.04
20	Perrilla aldehyde	19.14	1252.1	74.71	58.84
21	Bornyl acetate	19.81	1270.2	0.06	0.09
22	Perilla alcohol	20.10	1282.1	0.62	0.53
23	Methyl perillate	23.10	___	1.48	1.56
24	β -Copaene	23.49	1427.3	___	0.04
25	Caryophyllene	24.31	1419.3	___	0.02
26	γ-Decalactone	24.83	1426.9	0.17	0.12
27	β-Copaene	26.10	1427.3	___	0.06
28	Curcumene	26.16	1471.4	0.02	___
29	Bicyclogermacrene	26.57	1489.8	___	0.08
30	β-Cadinene	27.40	1513.9	___	0.04
31	D-Germacren-4-ol	28.72	1568.3	___	0.03
32	R-Turmerone	30.87	___	0.05	___
33	α-Cadinol	30.79	1640.2	___	0.08
34	Shyobunol	31.72	1592.8	0.05	0.46
35	Chamazulene	32.51	1710.0	0.04	0.08
The total identified components				**99.51**	**99.83**
Monoterpenoids hydrocarbons				**21.68**	**37.67**
Oxygenated monoterpenoids				**77.71**	**61.35**
Sesquiterpenoids hydrocarbons				**0.02**	**0.24**
Sesquiterpenoids monoterpenoids				**0.1**	**0.57**

GC–MS = Gas chromatography–mass spectroscopy. RT = Retention time. RI= retention indices in DIMS—dimethylsilicone as Stationary phase.

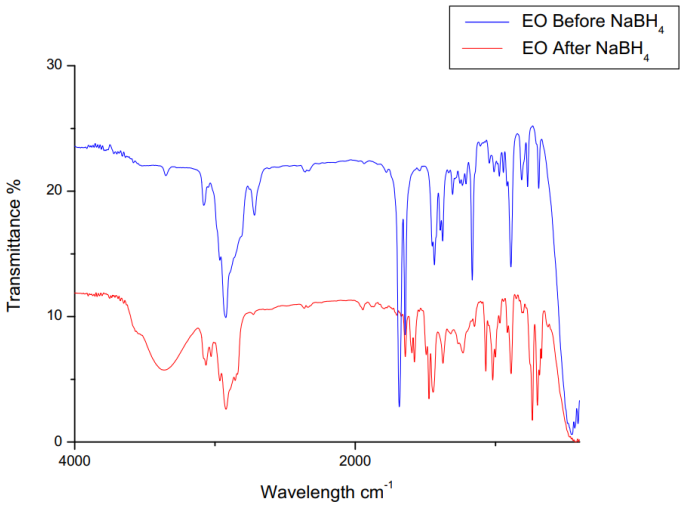


Figure 4 The IR spectra of the hemisynthetic perilla alcohol (a)

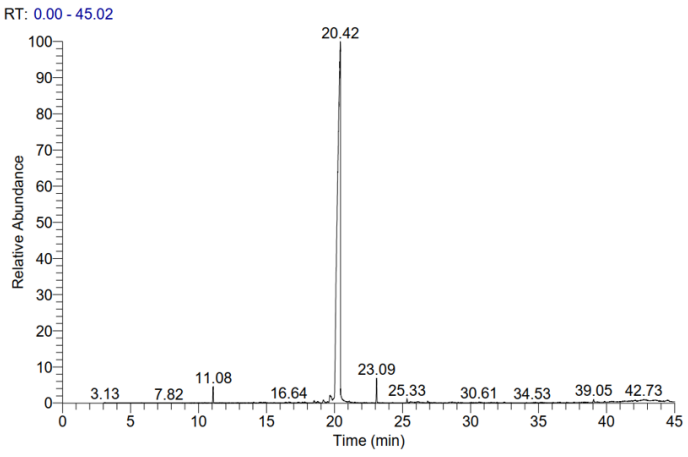
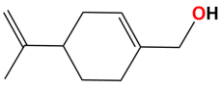


Figure 5 The chromatogram of the hemisynthetic perilla alcohol (a)

Table 4 GC–MS analysis of EO after reduction with $NaBH_4$.

Components	RT	RSI	Percentage area %	Chemical structure
Perilla alcohol	20.42	935	94.65	

GC–MS = Gas chromatography–mass spectroscopy. RT = Retention time. RSI: The Reverse Match Factor (RSI > 600)

The disproportionation of Perillaldehyde reaction was done in a strongly basic solution. A white precipitate was obtained and the solid was washed with Hexane and diethyl ether to remove the impurities. The yield was 42.85%. The m.p was confirmed (60°C) then analyzed by IR: 3400, 2917, 2842, 1692, 1417 et 1229 cm^{-1} (see figure 6). according to the spectrum the strong broad band around 3300 cm^{-1} correspond to OH-stretching of carboxylic acid and the strong band around 1692 cm^{-1} correspond to CO stretching of carboxylic acid and the medium band around 1417 cm^{-1} correspond to OH-bending.
The obtained product was analyzed also by GC-Ms. The comparison of the mass spectra obtained for the sample and those of the built-in libraries NIST, showed the match of the samples chromatogram with the perillic acid chromatogram (b) (see figure 7).

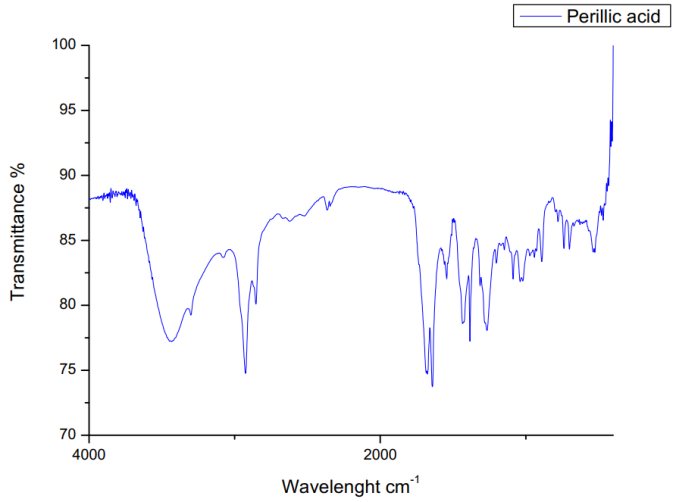


Figure 6 The IR spectra of the hemisynthetic perillic acid (b)

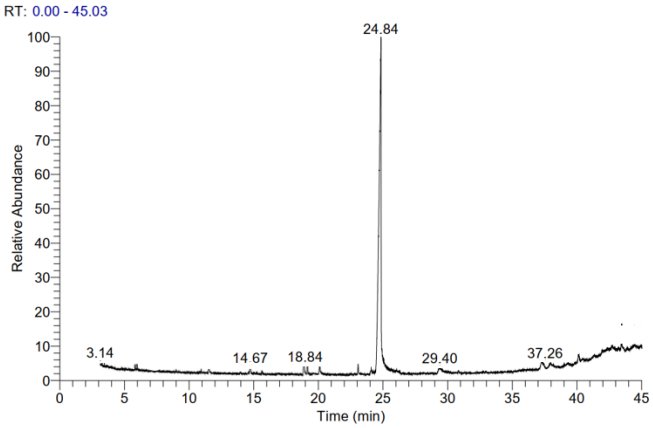
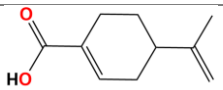


Figure 7 The chromatogram of the hemisynthetic perillic acid (b)

Table 5 GC–MS analysis of Perillic acid.

Components	RT	RSI	Percentage area %	Chemical structure
Perillic acid	24.85	927	71.2	

GC–MS = Gas chromatography–mass spectroscopy. RT = Retention time. RSI: The Reverse Match Factor (RSI > 600).

The reaction of perillaldehyde oxime offered a white solid with a yield of 92.85% that was purified. The purity of recrystallized oxime has been confirmed by the melt point and the IR spectra and the physical data of Perillartine (c): m.p 102°C. IR: 3427, 2917, 2361, 1643, 630 cm^{-1} (see figure 8). according to the spectrum the strong broad band around 3400 cm^{-1} correspond to OH-stretching of oxime and the medium band around 1643 cm^{-1} correspond to CN-stretching of oxime.

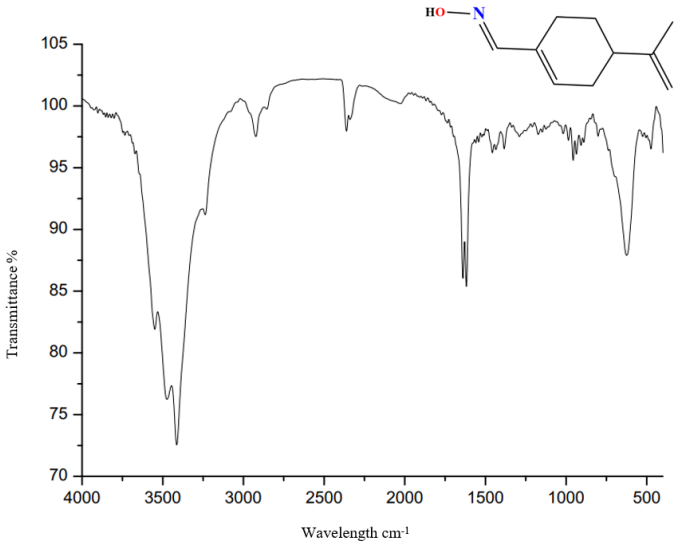


Figure 8 the IR spectra of the hemisynthetic perillartine (c)

CONCLUSION

The molecular docking study of the three compounds Perilla alcohol, perillic acid, and perillartine has shown significant anticancer activity against lung cancer. Considering their good binding affinities with lung cancer receptors and their good oral bioavailability as good absorbed drugs according to Lipinski's rule. Therefore, those compounds could be used in the pharmaceutical industries against lung cancer.
The objective of the comparative study on the chemical composition of *Ammodaucus leucotrichus* Coss, essential oils from two regions in Morocco Errachidia and Smara, was to determine which essential oil could be exploited in the medicinal and pharmaceutical industry as a potent source of perillaldehyde. The results of this study showed that the essential oil from the Errachidia region was obtained with a higher yield of 4.26% and GC-Ms analysis of both essential reveals that the EO from the Errachidia region is very abundant in perillaldehyde with a percentage of 74.71%. The semisynthetic compounds perilla alcohol, perillic acid, and perillartine were obtained with good yields. Therefore, using semi-synthesis instead of bioconversion is more rentable.
At the end of this study, *Ammodaucus leucotrichus* essential oil can be recommended as valuable starting material leading to active molecules with anticancer and properties and exploited in the medicinal industry.

Acknowledgments: Teacher Laura Santi from the American Language Center Meknes in Morocco for the language editing.
The common analysis center of the faculty of sciences Meknes in Morocco.

Conflict of interests: We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

Abu Zarga, M.H., Al-Jaber, H.I., Baba Amer, Z.Y., Sakhrib, L., Al-Qudah, M.A., Al-humaidi, J.Y.G., Abaza, I.F., Afifi, F.U., (2013). Chemical Composition, Antimicrobial and Antitumor Activities of Essential Oil of Ammodaucus leucotrichus Growing in Algeria. *Journal of Biologically Active Products from Nature*. from Nat. 3, 224–231. https://doi.org/10.1080/22311866.2013.833469.
Babushok, V.I., Linstrom, P.J., Zenkevich, I.G., 2011. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *J. Phys. Chem.* Ref. Data 40. https://doi.org/10.1063/1.3653552
Bhuyar, P., Rahim, M. H. A., Maniam, G. P., & Govindan, N. (2021). Isolation and characterization of bioactive compounds in medicinal plant centella asiatica and study the effects on fungal activities. *Journal of Microbiology,Biotechnology and Food Sciences*, 10 (4), 631-635 https://doi.org/10.15414/jmbfs.2021.10.4.631-635
C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 2001. 46.
Daina, A., Michielin, O., Zoete, V., (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*. 7, 1–13. https://doi.org/10.1038/srep42717.
El-Haci, I.A., Bekhechi, C., Atik-Bekkara, F., Mazari, W., Gherib, M., Bighelli, A., Casanova, J., Tomi, F., (2014). Antimicrobial activity of Ammodaucus

leucotrichus fruit oil from Algerian Sahara. *Natural Product Communications*. 9, 711–712. https://doi.org/10.1177/1934578x1400900533.

Gogoi, R., Loying, R., Sarma, N., Munda, S., Kumar, S., (2018). Industrial Crops & Products A comparative study on antioxidant , anti-in fl ammatory , genotoxicity , anti- microbial activities and chemical composition of fruit and leaf essential oils of Litsea cubeba Pers from North-east India. *Industrial Crops & Products journal*. 125, 131–139. https://doi.org/10.1016/j.indcrop.2018.08.052.

Gurnani, N., Mehta, D., Gupta, M., Mehta, B.K., (2014). Natural Products : Source of Potential Drugs Natural Products Lab , *African Journal of Basic & Applied Sciences*, 6, 171–186. https://doi.org/10.5829/idosi.ajbas.2014.6.6.21983.

Imamura, M., Sasaki, O., Okunishi, K., Nakagome, K., Harada, H., Kawahata, K., Tanaka, R., Yamamoto, K., Dohi, M., (2014). Perillyl alcohol suppresses antigen-induced immune responses in the lung. *Biochemical and Biophysical Research Communications*. 443, 266–271. https://doi.org/10.1016/j.bbrc.2013.11.106.

Lal, M., Borah, A., Pandey, S.K., (2019)a. Identification of a New High Geraniol Rich Variety "Jor Lab L-15" of Lemongrass [Cymbopogon khasianus (Hack) Stapf (ex Bor)]. *Journal of Essential Oil Bearing Plants.* TEOP 22 (4) 972 - 978. https://doi.org/10.1080/0972060X.2019.1661797

Lal, M., Dutta, S., Munda, S., Pandey, S.K., (2019)b. Identification and Registration of a High Essential Oil Yielding Variety (Jor Lab L-14) of Lemongrass (Cymbopogon flexuosus L .) Through Mutation Breeding Technique. *Journal of Essential Oil Bearing Plants* TEOP 21 (6) 2018 pp 1604 - 1611. https://doi.org/10.1080/0972060X.2018.1559105.

Manssouri, M., Znini, M., El Harrak, A., Majidi, L., (2016). Antifungal activity of essential oil from the fruits of Ammodaucus leucotrichus Coss. & Dur., in liquid and vapour phase against postharvest phytopathogenic fungi in apples. *Journal of Applied Pharmaceutical Science* Vol.. 6, 131–136. https://doi.org/10.7324/JAPS.2016.60520.

Manssouri, M., Znini, M., Majidi, L., (2020). Studies on the antioxidant activity of essential oil and various extracts of Ammodaucus leucotrichus Coss. & Dur. Fruits from Morocco *Journal of Taibah University for Science*. 14, 124–130. https://doi.org/10.1080/16583655.2019.1710394.

Naczk, M., Shahidi, F., (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*. 41, 1523–1542. https://doi.org/10.1016/j.jpba.2006.04.002.

Noudjou, F., Kouninki, H., Ngamo, L.S.T., Maponmestsem, P.M., Ngassoum, M., Hance, T., Haubruge, E., Malaisse, F., Marlier, M., Lognay, G.C.,(2007). Effect of site location and collecting period on the chemical composition of hyptis spicigera lam. An insecticidal essential oil from north-Cameroon. *J. Essent. Oil Res*. 19, 597–601. https://doi.org/10.1080/10412905.2007.9699340

Prakash, O., Kumar, A., Kumar, P., Ajeet, A., (2013). Anticancer Potential of Plants and Natural Products: A Review. *American Journal of Pharmacological Sciences* Vol. 1, No. 6, 104-115. https://doi.org/10.12691/ajps-1-6-1.

Purcell WP, Singer JA. (1967) A brief review and table of semiempirical parameters used in the Hueckel molecular orbital method. *J Chem Eng Data*;12(2):235-46. https://doi:10.1021/je60033a020.

Setamdideh, D., Rafigh, M., (2012). Reduction of carbonyl compounds to their corresponding alcohols by (Nicotine) (tetrahydroborato)zinc complex as a new stable and efficient reducing agent. *E-Journal Chem*. 9, 2338–2345. https://doi.org/10.1155/2012/391643.

Swain, S.K., Chandra Dash, U., Kanhar, S., Sahoo, A.K., (2020). Ameliorative effects of Hydrolea zeylanica in streptozotocin-induced oxidative stress and metabolic changes in diabetic rats. *J. Ethnopharmacol*. 247, 112257. https://doi.org/10.1016/j.jep.2019.112257.

Torre, L.A., Siegel, R.L., Jemal, A., (2016). Lung cancer statistics. *Advances in Experimental Medicine and Biology* 893, 1–19. https://doi.org/10.1007/978-3-319-24223-1_1.

T. J. Raphael and G. Kuttan, (2003). Immunomodulatory Activity of Naturally Occurring Monoterpenes Carvone, Limonene, and Perillic Acid. *Immunopharmacology and immunotoxicology* Vol. 25, No. 2, pp. 285–294, https://doi.org/10.1081/IPH-120020476.

Yadav, D.K., Kumar, S., Saloni, Misra, S., Yadav, L., Teli, M., Sharma, P., Chaudhary, S., Kumar, N., Choi, E.H., Kim, H.S., Kim, M.H., (2018). Molecular Insights into the Interaction of RONS and Thieno[3,2-c]pyran Analogs with SIRT6/COX-2: A Molecular Dynamics Study. *Scientific Reports* . 8, 1–16. https://doi.org/10.1038/s41598-018-22972-9.

Yeruva, L., Pierre, K.J., Elegbede, A., Wang, R.C., Carper, S.W., (2007). Perillyl alcohol and perillic acid induced cell cycle arrest and apoptosis in non small cell lung cancer cells. *Cancer Letters* 257 216–226. https://doi.org/10.1016/j.canlet.2007.07.020.

Yun, C.H., Boggon, T.J., Li, Y., Woo, M.S., Greulich, H., Meyerson, M., Eck, M.J., (2007). Structures of Lung Cancer-Derived EGFR Mutants and Inhibitor Complexes: Mechanism of Activation and Insights into Differential Inhibitor Sensitivity. *Cancer Cell* 11, 217–227. https://doi.org/10.1016/j.ccr.2006.12.017.