

## Phytochemical and pharmacological study of four aromatic plants growing in northeast of Algeria

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### ABSTRACT/RESUME

**Abstract:** *Calamintha baborensis*, (Lamiaceae) is used in folk medicine as a stomachic and antidiarrhetic, however, *Lavandula stoechas* (Lamiaceae) is used as an expectorant, carminative and against flu and asthma. *Myrtus communis* (Myrtaceae) and *Pistacia lentiscus* (Anacardiaceae) both are used as stomachic. Comparison of the chemical composition and evaluation of the antioxidant capacity of the plants essential oils, and their acute toxicity were carried out in this study. The essential oils were analyzed by GC-MS and the free radical scavenging activity of essential oils was evaluated using DPPH test. In vivo pharmacological oxidative stress assays were carried out on male albino Swiss mice. The lipid peroxidation was assessed as the generation of thiobarbituric acid-reactive substances (TBARS) followed by glutathione (GSH) measurement. The main constituents of *C. baborensis* oil were isomenthone (67.44%), pulegone (15.07%) and piperitone oxide (6.53%). In *L. stoechas* oil, the main constituents were L-fenchone (47.05%), eucalyptol (13.23%), camphor (11%), 3-carene (3.57%) and  $\alpha$ -pinene (3.29%). *M. communis* has as major compounds 1,8-cineole (27.85%),  $\alpha$ -pinene (15.42%), D-limonene (12.87%) and benzene, 1-methyl-2-(1-methylethyl)- (9.63%) and 1,6-octadien-ol, 3,7-dimethyl-, acetate, (Z)- (3.26%). *P. lentiscus* has O-cymene (22.94%), Menthone (12.09%),  $\beta$ -pinene (10.35%),  $\alpha$ -pinene (9.41%),  $\alpha$ -Farnesene (9.22%),  $\beta$ -myrcene (7.69%), Trans-pinocarveol (6.86%), Limonene (6.34%) and Pulegone (5.29%) as major constituents. The screening of possible in vitro antioxidant activity of essential oils by DPPH free radical-scavenging test showed a reduction in scavenging activity of essential oils of *C. baborensis* (50.35-88.93%), *L. stoechas* (55.37-86.77%), *M. communis* (42.14-54.54%) and *P. lentiscus* by (53.71-76.03%). Furthermore, essential oils have caused an increase in MDA and reduced glutathione in the liver as well as in the lungs of treated animals compared to controls. The results showed that essential oils of *C. baborensis*, and *L. stoechas*, *P. lentiscus* and *M. communis* belonging to the chemotypes isomenthone, fenchone, O-cymene and 1,8-cineole, respectively, are powerful antioxidant but could be prooxidant at high doses.

## I. Introduction

Oxidative stress is caused by the generation of free radicals and reactive oxygen species (ROS) which damage the cellular macromolecules [1]. The presence of increased amount of ROS resulting in cellular and systemic dysfunction [2]. Excessive production of the ROS and the oxidative stress play an important role in various health problems such as ageing, arteriosclerosis, cancer, Alzheimer's disease, Parkinson's disease, diabetes and asthma [3-4]. ROS are continuously produced by eukaryotic cells [1]; and to maintain a cellular balance between ROS generation and clearance, humans have developed highly complex antioxidative defense mechanisms, it involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically, including enzymes and antioxidants [4-5]. However, the innate defense systems of body may be supported by antioxidative compounds taken as foods [6]. Although, the most widely used antioxidants are synthetic antioxidants such as butylated hydroxytoluene. Hence, the development of alternative antioxidants from natural sources has considerable prospects [7, 8].

Phytonutrients or phytochemicals, substances produced by plants, are becoming increasingly known for their antioxidant activity [9]. This antioxidant activity is clearly associated with the activity of "free radical scavenging enzymes" (superoxide dismutase, catalase, peroxidase, etc.) and the contents of antioxidant substances are mainly phenolic compounds, carotenoids, tocopherol and ascorbic acid [9-10]. Medicinal plants continue to be a source of remedies, and natural products are an inspiration for new medicine [11]. In the Mediterranean areas medicinal plants have important antioxidant properties with many potential pharmaceutical applications [12]. Furthermore, many studies reported that aromatic plants and their essential oils possessed strong antioxidant activities. *Pistacia lentiscus* L. and *Myrtus communis* L. are promising sources of natural antioxidants [13-14]. Antioxidant activities are also confirmed for most of the phenolic compounds present in different spices and herbs, and their different chemical composition in general, especially in the Lamiaceae family [15-19].

It is well known that genotypes, season of collection, genetic factors and geographical origin have a considerable effect on plant oils composition. The altitude can also be considered as a major factor influencing the physiological and chemical responses of plants [20-24]. *Calamintha* and *Lavandula* are two closely related genera, both belonging to the family Lamiaceae. In Algeria, Lamiaceae family includes about 28 genera and 04 sub-genera. Five species of *Calamintha* genus can

be found, one of them is *Calamintha baborensis* (Batt.) Briq. The genus *Lavandula* comprise five species one of them is *Lavandula stoechas* L. Two (2) species of *Myrtus* genus [25-26] and three (3) species of *Pistacia* genus [26-27] were found. *C. baborensis* is used in folk medicine as a stomachic and antidiarrhetic. However, *L. stoechas* infusion is used in Tunisia as an expectorant, carminative and against flu and asthma [28, 29]. *M. communis* is used in traditional medicine as hypertensive agent and as additional resources for natural antioxidant [30]. In traditional medicine of Morocco, *P. lentiscus* is used as stomachic [31]. Furthermore, in Algeria, its oil is used as an anti-inflammatory, healing and eye drops. *Calamintha* and *Lavandula* species have been studied mainly for their essential oil contents [32-37]. As an important form of alternative medicine, aromatherapy principles should be introduced to pharmacy to prepare a new generation of healthcare drugs and professionals [38]. Therefore, the main objectives of this study were:

- (i) to determine the chemical composition of steam distilled oils of the aerial parts of the four species growing in Texanna - Algeria by gas chromatography/mass spectrometry (GC-MS).
- (ii) to evaluate the antioxidant capacity of the plant essential oils.
- (iii) to evaluate their acute toxicity.

## II. Materials and methods

### II.1. Plant material

The aerial part of each sample was collected. *C. baborensis* were collected during the flowering stage in January 2013 from Texanna, Algeria, (36° 38' 10,91" north latitude 5° 47' 53,61" longitude east) in late flowering stage. *L. stoechas* were collected in May 2013 at the same region (36° 39' 89" north latitude 5° 47' 52" longitude east). *M. communis* (43° 36' 57,19" north latitude 47° 5' 16,53" longitude east) and *P. lentiscus* (43° 36' 19,05" north latitude 46° 5' 54,02" longitude east) were collected in June 2014. The species were identified by Dr Mohammed Bouldjedri and a voucher sample was deposited in the herbarium National Parc of Taza (Jijel) under code numbers LSC12, LL1, MM1 and AP1, respectively.

#### II.1.1. Extraction of essential oils

The dried plant material (100 g) of the four samples was extracted by steam distillation using Clevenger-type apparatus for 3.5 h. The oil was dried under anhydrous sodium sulphate and stored at 3°C until analysis.

### II.1.2. GC-MS Analysis

The identification of the components was performed based on their retention indices (RI) and gas chromatography coupled to mass spectrometry (GC-MS). The gas chromatograph (*Schimadzu* QP 2010) is coupled to a quadruple mass spectrometer type EI model 70 eV equipped with an apolar capillary column SE30. The temperature of the split injector was 250 °C. The injector was programmed from 40 °C (10 min) to 220 °C at 5 °C/min and was maintained at 220 °C for 5 min. Transfer line was maintained at 250 °C. Carrier gas: He at 2.0 ml/min (The mean linear velocity 35 cm/sec). The temperatures of the source and the interface were set to 200 °C and 250 °C, respectively mass range 40 to 450 amu. The compounds were identified by the NIST library 05.

### II.1.3. Free radical scavenging assay

The free radical scavenging activity of essentials oils was evaluated using the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This method was adapted from the work of Brand-Williams, Cuvelier and Berset (1995) with some modifications: 15 µl of each oil test was twofold diluted to different concentrations (0.01, 0.1 and 0.5 mg/ml) with methanol and was mixed with 1.5 ml DPPH in methanol. The absorbance of the remaining DPPH was determined during 30 minutes at 515 nm. Blank sample contained the same amount of ethanol and DPPH. The measurements were performed in duplicate. The radical scavenging activity or antioxidant activity (%) of each concentration of oil was calculated using the following formula:

$$\text{DPPH activity (\%)} = [(Odc - Ods)/(Odc)] \times 100\%.$$

(*Odc*: Optical density of control. *Ods*: Optical density of the samples) [39].

## II.2. Animals

The animals used in this study were male albino Swiss mice of an average weight of about 23 g. The animals were kept into polystyrene cages having free access to food and water. The weight variation of animals was followed throughout the treatment period. 27 mice were used in this study and were divided into 9 groups of 3 animals each:

- Group 1 (control) served as control receiving by gavage 0.5 ml of water (vehicle for dissolution of essential oils for 3 days).
- Group 2 (*C. baborensis* orally) received orally essential oil of *C. baborensis* (40 mg/kg/day) for three days.

- Group 3 (*C. baborensis* nasally) received nasally essential oil of *C. baborensis* (40 mg/kg/day) for three days.
- Group 4 (*L. stoechas* orally) received orally essential oil of *L. stoechas* (40 mg/kg/day) for three days.
- Group 5 (*L. stoechas* nasally) received nasally essential oil of *L. stoechas* (40 mg/kg/day) for three days.
- Group 6 (*M. communis* orally) received orally essential oil of *M. communis* (40 mg/kg/day) for three days.
- Group 7 (*M. communis* nasally) received orally essential oil of *M. communis* (40 mg/kg/day) for three days.
- Group 8 (*P. lentiscus* orally) received nasally essential oil of *P. lentiscus* (40 mg/kg/day) for three days.
- Group 9 (*P. lentiscus* nasally) received nasally essential oil of *P. lentiscus* (40 mg/kg/day) for three days.

The animals were sacrificed by cervical dislocation after three days of treatment. The liver and the lungs of each mouse were collected and washed in a solution of NaCl 9‰ and then stored at 20°C until analysis of cellular oxidative stress.

### II.2.1. Lipid peroxidation assessment

The generation of thiobarbituric acid- reactive substances (TBARS) was used to assess lipid peroxidation. Briefly, 0.2 g of lung or liver was mixed with 0.6 ml of KCl (1.15%). After the addition of 1 ml trichloroacetic acid (3%), and 1ml of thiobarbituric acid (TBA 1%), all tubes were heated at 95 °C for 30 min. Lipid peroxidation adducts were extracted by n-butanol and absorbance was measured at 532 nm. Results were expressed as nmol malondialdehyde (MDA)/g tissue [40-41].

### II.2.2. Glutathione (GSH) measurement

Portions (approximately 0.2 g) of liver or lung freshly excised or frozen were homogenized in three volumes of 5% TCA using Dounce homogenizer. The samples were centrifuged at 2000 rpm for 15 minutes and 50 µl of the supernatant were diluted in 10 ml phosphate buffer (0.1 M, pH 8.0). In addition, 20 µl of DTNB 0.01 M was added to 3 ml of the dilution mixture. The measurement was performed at 412 nm against a control prepared in the same conditions using 5% TCA. The concentrations were expressed in mmoles of GSH/g of liver or lung. They were deduced based on a standard curve of GSH, which was prepared in the same conditions cited above [41-42].

### II.3. Statistical analyses

Data were analyzed by ANOVA (STATISTICA 7) and are expressed as mean  $\pm$  standard deviation.

### III. Results and discussion

The yield obtained by steam distillation of the aerial parts of *C. baborensis*, *L. stoechas*, and *P. lentiscus* was 1.04%, 0.60%, and 0.27% of yellowish oils, respectively, whereas the yield of *M. communis* was 0.42% of greenish oil. In a previous study, the aerial parts of *C. hispidula* growing in El-Milia-Algeria [43], gave 0.35%, and the aerial parts oils of *L. stoechas* varies from 0.77-1.2% [44]. In the essential oil extracted from *C. baborensis*, 17 compounds were identified by GC/MS analysis; their predominant constituents were isomenthone (67.44%), pulegone (15.07%) and piperitone oxide (6.53%), with a high proportion of oxygenated

monoterpenes (Table 1). The highest amount of isomenthone and pulegone were noticed in several species with different percentages. In *C. grandiflora* growing in Greece the percentage of isomenthone was 15.2% and pulegone was 35.2% [45], while in that of France, isomenthone and pulegone were found at the concentrations of 24.7% and 27.6%, respectively [46]. In the oil isolated from *C. origanifolia* (Lebanon flora), isomenthone and pulegone were found at the concentrations of 15.1% and 7.4%, respectively [37] and varied for *C. nepeta* between (0.6-24.4%) and (17-68%). The antioxidant activity of pulegone, neo-menthol and isomenthone was (39.5%), (33%) and (19.6%), respectively, of the essential oil of *Satureja calamintha* evaluated by DPPH free radical-scavenging and reducing power [47].

**Table 1.** Chemical composition of essential oils of *Calamintha baborensis* (Batt.) Briq.

Compounds	Percentage %	RI
$\alpha$ -pinene	0.65	972
$\alpha$ -phellandrene	0.58	992
$\beta$ -pinene	1.30	993
$\beta$ -myrcene	0.85	1005
1,3,8-p-menthatriene	0.15	1027
Cyclobutane, 1,2-bis(1-methylethenyl)-,trans-	2.61	1029
Ocimene	0.86	1038
$\gamma$ -terpinene	0.24	1052
$\alpha$ -thujone	0.92	1055
L-Fenchon	0.14	1065
Menthol	0.74	1125
Isomenthone	67.44	1140
trans-Dihydrocarvone	0.79	1149
4-terpinenol	0.74	1162
Pulegone	15.07	1254
Piperitone oxide	6.53	1284
Caryophyllene	0.33	1445

Regarding *L. stoechas*, 43 compounds were identified; oxygenated monoterpenes fraction was predominant in comparison with sesquiterpenes fraction. It contained L-fenchone (47.05%), eucalyptol (13.23%), camphor (11%) and 3-carene (3.57%) as major constituents (Table 2). The monoterpene ketone (L-fenchone) is generally known as the main characteristic components of the oils of the *Lavandula* species. In the aerial parts of *L. stoechas*, L-fenchone (32.03%) and camphor (14.71%) were identified as the main constituents [33]. Furthermore, the essential oil of *L. stoechas* collected in Greece was characterized by high contents of fenchone (45.19%), 1,8-cineole (16.30%) [34] whereas the aerial part oils of *L.*

*stoechas* growing in Morocco was characterized by low quantity of D-fenchone (3.06%) [35].

*P. lentiscus* oil is characterized by 12 compounds, the majority of which are o-cymene (22.94%), menthone (12.09%),  $\beta$ -pinene (10.35%),  $\alpha$ -pinene (9.41%),  $\alpha$ -farnesene (9.22%),  $\beta$ -myrcene (7.69%), trans-pinocarveol (6.86%), limonene (6.34%), pulegone (5.29%) and verbenone (4.45%) (Table 3). The essential oil of *P. lentiscus* is constituted mainly of myrcene (15.18%) and 1,8-cineole (15.02%) [48].  $\alpha$ -pinene and  $\beta$ -pinene are known as compounds with antimicrobial potential. However, the antifungal activity of essential oil of *P. lentiscus* is due to the  $\alpha$ -pinene which constitutes a considerable amount of this oil [49].

**Table 2.** Chemical composition of essential oils of *Lavandula stoechas* L.

Compounds	Percentage %	RI
cyclohexene 4-methylene-1-(1-methylethyl)-	0.12	952
$\alpha$ -pinene	3.29	956
Camphene	2.51	961
Benzene, tert-butyl-	0.09	969
$\alpha$ -thujene	0.1	971
Sabinène	0.31	972
$\beta$ -Myrcene	0.18	981
3-Carene	3.57	990
1,3,8-p-Menthatriene	0.41	994
Eucalyptol	13.23	998
Fenchone	47.05	1028
(+)-4-Carene	0.21	1037
Linalool	1.04	1044
Fenchol, exo-	0.43	1050
Campholenic aldehyde	0.16	1055
Camphor	11	1064
(S)-cis-Verbenol	0.37	1074
Methyldihydrojasmonate	0.39	1082
Isobornyl formate	0.61	1088
benzenemethanol .alpha. .alpha. 4-trimethyl-	0.43	1098
4-tepinenol	0.72	1099
2-Pinene-10-methyl acétate	0.26	1101
$\alpha$ terpinéol	0.28	1112
Myrtenol	0.86	1119
Fenchyl acetate	0.31	1150
Carvone	0.14	1158
Isobornyl acetate	3.09	1242
(-)-Myrtenyl acetate	3.14	1313
$\alpha$ -Cubebene	0.16	1358
ethanone, 1-cyclopropyl-2-(4-pyridyl)-	0.05	1370
(E)-beta-Farnesene	0.06	1401
5,10-Pentadecadyne, 1-chloro-	0.05	1425
Isoledene	0.21	1448
9-methylene[4.4.0]dec-1-ene	0.12	1462
naphthalene 1 2 3 4 4a 5 6 8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene	0.37	1472
Longipinene	0.38	1483
Cis- $\alpha$ -Copaene-8-ol	0.36	1497
1,9-Aristoladiene	0.1	1528
Aromadendrene	0.51	1542
Alloaromadendrene	0.12	1571
bicyclo [4,4,0] dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.9	1804
Andrographolide	0.34	1806

The essential oil of *M. communis* contains 32 compounds, the major constituents were 1,8-cineole (27.85%); Alpha-pinène (15.42%); D-Limonene (12.87%); Benzene,1-methyl-2-(1-methylethyl)- (9.63%) and 2,6-Octadien-ol, 3,7 -dimethyl-,acetate, (Z)- (3.26%) (Table 4). According to other researches that studied the essential oils of *M. communis*, it showed that oils

from France, are mainly consist of 1,8-cineole (eucalyptol) and alpha-pinene [50]. The volatile oil in leaves of *M. communis* L. growing in Turkey contains 1,8-cineole, linalool, myrtenyl acetate and myrtenol as major components [51-53]. Those of Tunisia have as major constituents alpha-pinene (45.9 and 51.29%), 1,8-cineole (19 and 23.1%) and limonene (9 and 9.7%) [54].

**Table 3.** Chemical composition of essential oils of *Pistacia lentiscus* L.

Compounds	Percentage %	RI
$\alpha$ -pinene	9.41	972
$\beta$ -pinene	10.35	993
$\beta$ -myrcene	7.69	1004
O-cymene	22.94	1022
Limonene	6.34	1029
Hexanoic acid 2-methyl-	3.19	1111
Trans-pinocarevol	6.86	1115
Menthone	12.09	1139
4-terpineol	2.17	1163
Verbenone	4.45	1170
Pulegone	5.29	1302
$\alpha$ -Farnesene	9.22	1350

The essential oils of *M. communis* contains mainly alpha-pinene (15-57%), 1,8-cineole (12 to 45%), limonene (5 to 19%), linalool (2 to 19%), myrtenol (0.7-5%) and myrtenyl acetate (1-35%) [55]. Finally, the essential oil of myrtle (*M. communis*) rarely used in pharmacy, contains the myrtenyl

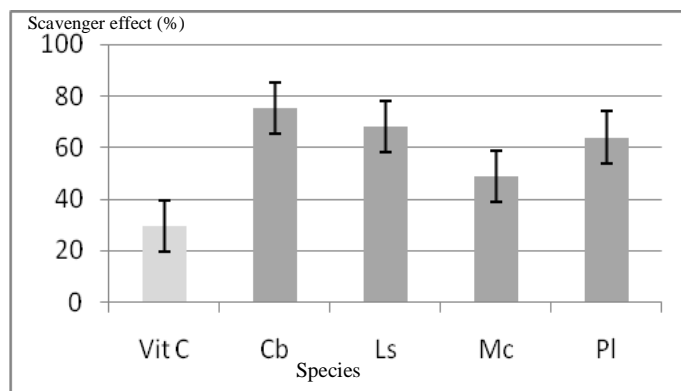
acetate,  $\alpha$ -pinene, cineole, myrtenol, linalool, methyl eugenol, etc. [56]. The essential oils of Algerian *M. communis* consisted mainly of monoterpenes,  $\alpha$ -pinene (27.4–59.2%) and 1,8-cineole (6.1–34.3%)[57].

**Table 4.** Chemical composition of essential oils of *Myrtus communis* L.

Compounds	Percent%	RI
$\alpha$ - pinene	15.42	972
$\beta$ - pinene	1.73	993
$\beta$ -Myrcene	0.36	1005
$\alpha$ -Phellandrene	1.55	1112
Carene	2.31	1117
Benzene, 1-methyl-2-(1-methylethyl)-	3.26	1024
Eucalyptol	27.85	1030
D-Limonene	12.87	1032
Ocimène	1.2	1047
Terpinene	2.77	1053
Fenchone	0.5	1065
$\delta$ -2-Carene	1.7	1077
Linalol	2.44	1086
isovalerate	0.97	1092
D-Camphor	0.19	1110
Trans-Pinocarveol	0.19	1115
Terpinen-4-ol	0.25	1163
$\alpha$ -Terpinole	2.02	1178
Estragole	0.44	1183
Geraniol	0.92	1315
Linalyl acetate	1.05	1319
$\alpha$ -Terpineol	2.43	1399
Copaene	0.14	1422
2,6-Octadien-ol, 3,7 -dimethyl-,acetate, (Z)-	9.63	1425
Methyl eugenol	1.92	1429
$\beta$ -Elemene	0.7	1432
Caryophyllene	1.83	1446
$\alpha$ -Caryophyllene	0.74	1467
Selinene	0.41	1487
Selina-3,7(11)-dien	0.36	1495
2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl	0.41	1505
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7- (1-methylethenyl)	0.42	1555
Caryophyllene oxide	0.61	1576

Figure 1 shows that the anti free radical activities of the four species essential oils are significantly higher ( $p < 0.05$ ) than that of the vitamin C (standard molecule). Indeed, with the concentrations of 0.5; 0.1 and 0.01 mg/ml the antioxidant activity of oils of all species are largely higher than that of the control (vitamin C) at the same concentrations (86.62% for *C. baborensis*

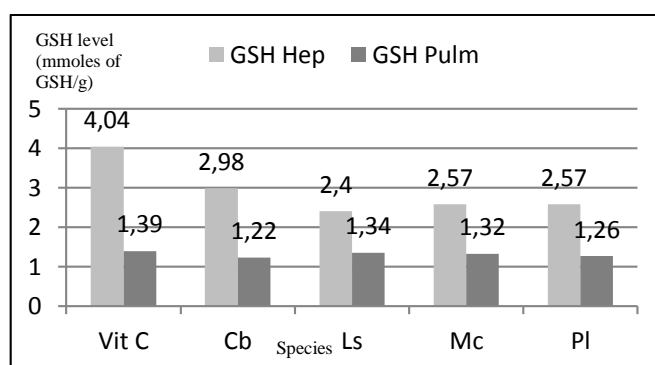
against 13.86% for the vitamin C). The total antioxidant activity is dose-dependent for vitamin C as well as for the tested essential oils; however, the *C. baborensis* oil had greater scavenging activity against DPPH. Compared with ascorbic acid and butylated hydroxytoluene, the methanolic solutions of the essential oils have a high antioxidant capacity [58].



**Figure 1.** Scavenger effect of Vit C: Vitamine C and essential oils of; Cb: *Calamintha baborensis* (Batt. Briq.), Ls: *Lavandula stoechas* L., Mc: *Myrtus communis* L., Pl: *Pistacia lentiscus* L.

Concerning glutathione and comparing with animals treated by the ascorbic acid (vitamin C), we show a reduction of hepatic GSH by (-36.4%), -26.34%, and -40.6%, in the mice treated respectively, by essential oils of both species (*M. communis* and *P. lentiscus*), *C. baborensis* and *L.*

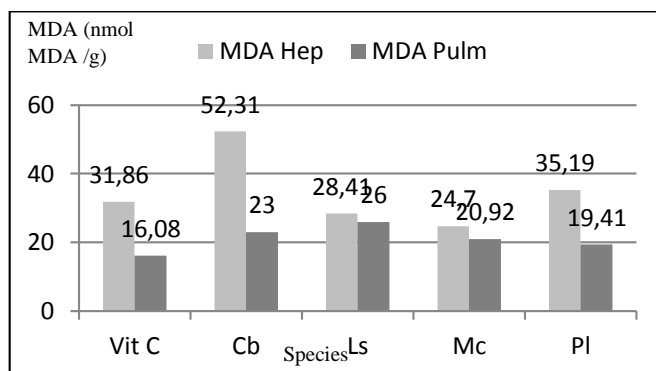
*stoechas* after 72 hours. After inhalation of essential oils during 3 days, we observed a low reduction in the pulmonary GSH, -5.035%, -9.352%, -12.23% and -3.597% for essential oils of *M. communis*, *P. lentiscus*, *C. baborensis*, and *L. stoechas*, respectively (Figure 2).



**Figure 2.** Variation of the rates of hepatic (Hep) and pulmonary (Pulm) GSH level (mmoles of GSH/g) after administration of 40 mg/kg/day during 72 hours. Cb: *Calamintha baborensis* (Batt. Briq.), Ls: *Lavandula stoechas* L., Mc: *Myrtus communis* L., Pl: *Pistacia lentiscus* L.

Figure 3 showed that the treatment by the essential oil of *C. baborensis* increased the cellular MDA as well in the liver (+64.187% compared to the control group) and in the lung (43.03%). On the other hand, the treatment of mice by the oil of *L. stoechas*, *M. communis* and *P. lentiscus* increased the level of MDA only in lung (61.691%, 30.099%, and 20.708%, respectively). It was reported that

malathion administration was accompanied by an oxidative stress status assessed by an increase of MDA in the kidney and liver, this result suggests that *L. stoechas* essential oil exerted potential hepato- and nephroprotective effects against malathion-induced oxidative stress in mice; the beneficial effect of *L. stoechas* essential oil might be related, in part, to its antioxidant properties [59].



**Figure 3.** Variation of the rates of the hepatic (Hep) and pulmonary (Pulm) MDA (nmol MDA /g tissue) after administration by oral way of essential oil 40mg/Kg/day during 72 hours. Cb: *Calamintha baborensis* (Batt. Briq.), Ls: *Lavandula stoechas* L., Mc: *Myrtus communis* L., Pl: *Pistacia lentiscus* L.

The antioxidant activity of *L. stoechas*, by trapping free radicals, which are the principal cause of lipidic peroxidation, was reported [29, 60]. *P. lentiscus* L. and *M. communis* L. are promising sources of natural antioxidants [13] and this activity can be related to the presence of phenolic compounds [29]. Essential oils of *C. baborensis* swallowed by the mice have been a reduction more pronounced in the hepatic GSH than those of *L. stoechas*, *M. communis* and *P. lentiscus* whereas the use of essential oils of *Eucalyptus globulus*, containing mainly 1,8-cineole is potentially dangerous [61]. The pathological and Ultra-structural observations indicated that the target organs of 1,8-cineole toxicity were the liver and kidney [62]; knowing that *L. stoechas* contains the 1,8 cineole (13.23%). After inhalation of essential oils by the animals, the pulmonary GSH was especially reduced in the treatment by oils of *C. baborensis* containing pulegone (15.07%) which is able to be toxic. The rate of glutathione in the liver of the mice treated with toxic oil amounts of pennyroyal (*Mentha pulegium*) or of R-(+)-pulegone, decreased by 75% in three hours [61, 63]. Both plants, *P. lentiscus* L. and *M. communis* L. showed potent antioxidant properties in all tested periods, these findings confirm the potential uses of *P. lentiscus* L. and *M. communis* L. in food technology and medicine [13]. Moreover, these major compounds: menthone, cymene, pinene and fenhone are good and natural antioxidants [64-66]. The antioxidant activity might be due not only to radical scavenging activity of antioxidant but also their affinity of the antioxydants to the substrates [67]. Another effect was observed *in vivo*, showing that the antioxidant or pro-oxidant effect of essential oils depends on the used concentration. On the other hand, this toxicity observed in eukaryotic cells allows their use as bio-insecticide. Furthermore it is necessary to link this toxicity to a

possible interaction between these oils and mitochondria [22].

For all statistical tests, a  $p < 0.05$  (value of 0.008) or less was considered significant.

#### IV. Conclusion

The obtained results indicated that *C. baborensis* belonging to the chemotypes isomenthone, *L. stoechas* to fenchone, *M. communis* to eucalyptol and *P. lentiscus* to o-cymene. The essential oils of the four species are powerful antioxidants. According to the results of GSH and MDA, these oils present some toxicity with the used doses *in vivo*. It is important to apply these essential oils on an *in vivo* cancer model to evaluate their anti-tumor effects.

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