CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Journal home page:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

# COMPARISON OF DIFFERENT EXTRACTION METHODS FOR THE DETERMINATION OF *PITURANTHOS SCOPARIUS* ESSENTIAL OILS: CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

**Zahida Yasmina Hebbache[1]✉, Foudil-Cherif Yazid[2], Hichem Mohammedi[2], Ouahiba Belfadel[2], Fodili Mokhtar[1]**

*[1]Laboratory of Organic Chemistry and Natural Substances, University Ziane Achour, City 05 July Road of Moudjbara, BP 3117, Djelfa, Algeria.*
*[2]Laboratory of Functional Organic Analysis, Department of Organic Chemistry, Faculty of Chemistry, University of Sciences and Technology Houari Boumediene (USTHB), El Alia, BP 32, Bab Ezzouar, Algeria.*
*✉hebbachezahidayasmina@gmail.com*

https://doi.org/10.34302/crpjfst/2021.13.3.13

**Article history:**
Received:
15 June 2021
Accepted:
12 August 2021

**Keywords:**
*Pituranthos Scoparius; Hydrodistillation, Microwave; assisted extraction; Steam distillation: Anti-inflammatory activity; Antimicrobial activity.*

**ABSTRACT**

The essential oils of *Pituranthos Scoparius* obtained by hydrodistillation (HD), microwave assisted extraction (MAE) and steam distillation (SD) were investigated for their chemical components, oil yield diversity and microbial activity. As a result of this investigation, the anti-inflammatory activity of the essential oils is reported here for the first time. The essential oils extracted from *P. Scoparius* were analyzed by GC/MS. Sixty-three compounds were identified, representing (87.69 %) in HD, (82.45 %) in MAE, and (88.94 %) in SD of the essential oils' total compositions. The predominant compounds identified were Dillapiol (16.38-37.21%), α-Pinene (0.48-10.84%) and Myristicin (4.21-9.37%). The antimicrobial activity of the essential oils was Identified using the disk diffusion method against seven bacteria strains and two yeasts. An appreciable antimicrobial activity was observed against *sarcina lutea*, and weak activity was observed against *staphylococcus epidermidis*, *saccharomyces cerevisiae* and *candida albicans*. The anti-inflammatory activity of the essential oils was Identified using the carrageenan induced edema method. The essential oils extracted by HD, MEA and SD showed an anti-inflammatory activity comparable to Diclofenac. The results reveal that the method of extraction of *P. scoparius* influences the chemical composition and anti-inflammatory activity of the essential oils.

## 1. Introduction

The genus *Pituranthos* is a member of the Apiaceaes family and consists of more than 20 species (Ozenda, 2004). *P. Scoparius* (Coss & Dur) Benth & Hook is a plant endemic to the Saharan region of North Africa (Quezel and Santa, 1962) known as "Tattayet" in Tamahaq (Tuareg language) and "Guezzah" in Arabic.

*P. Scoparius* is a perennial aphyllous plant. The stems are 40-80 cm tall. The flowers, often with peduncle, white petals and marrow veins, are bunched in lateral umbels that are fairly spread out. However, due to the high temperature of the Central Sahara from which the plants used in this experiment were obtained, the species used did not contain leaves, flowers, or seeds (Quezel and Santa, 1962). *P. Scoparius* is used in traditional Algerian medicine for many purposes. In infusion, the aerial part is used as a remedy for digestive difficulties, diabetes, hepatitis, asthma and rheumatic diseases (Ould el hadj *et al.*, 2003; (Hammiche

and Maiza, 2006) and also as an additive flavoring (Boukef, 1986; Benchelah et al, 2000). The powdered stems are used against reptile bites (Benchelah et al., 2000; Abdellah and Sahki, 2004).

Previous research has shown that *P. Scoparius* is an important source of essential oils, with a considerable variation in major compounds depending on the part used, the plant maturity, the climate conditions and the extraction methods employed (Vernin *et al.*, 1999; Vérité and Nacer, 2003; Smaili *et al.*, 2011; Gourine *et al.,* 2011; Lograda *et al.*, 2013; Hammoudi *et al.*, 2015; Belkacemi *et al.*, 2015; Chikhoune *et al.*, 2016; Ksouri *et al.*, 2017; Malti *et al.*, 2018, Attia *et al.*, 2011). In addition, Sabinene and α-pinene were identified as major compounds of most of *P. Scoparius* essential oils (Smaili *et al*, 2011; Lograda *et al*, 2013; Chikhoune *et al.*, 2016; Malti *et al.*, 2018; Attia *et al*, 2011). However, α-pinene was not identified in samples from some Algerian Saharan regions (Ghardaia, Bechar and Tamenrasset) (Belkacemi *et al.*, 2015; Ksouri *et al.*, 2017; Malti *et al.*, 2018) and Sabinene had not been reported in other studies on the same species (Vernin *et al.*, 1999; Vérité and Nacer, 2003; Gourine *et al.,* 2011; Hammoudi *et al.*, 2015; Belkacemi *et al.*, 2015; Ksouri *et al.*, 2017). Alternatively, samples from Ghardaïa, Laghouat, Bechar, Tamenrasset and Batna were characterized by very high contents of limonene (Gourine *et al.,* 2011; Ksouri *et al.*, 2017; Malti *et al.*, 2018). Bornyl Acetate was the dominant component of *P. Scoparius* essential oils from the Oum El Bouaghi and Tamenrasset regions (Vernin *et al.*, 1999; Hammoudi *et al.*, 2015). Other compounds were reported only in some samples and with appreciable amounts, such as: 7-methoxy-3-methyl-1-H-isichromen-1-one and methyl propene in P. *Scoparius* from Ghardaia (Belkacemi *et al.*, 2015), 6- methoxyelemicine in samples from Ghardaïa, Bechar and Batna (Malti *et al.*, 2018), Apiol in samples from Oum El Bouaghi (Vernin *et al.*, 1999) and Δ-3-Carene only in the Tunisian essential oil (Attia *et al*, 2011). Other essential oils were also characterized by a high presence of Myristicin (Vérité and Nacer, 2003; Smaili *et al.*, 2011; Gourine *et al.,* 2011; Malti *et al.*, 2018). These reported results confirmed the existence of different chemotypes in *P. Scoparius* essential oils.

Several studies reported the significant effect of the extraction methods on the yields and the chemical compositions of essential oils (Lucchesi *et al.*, 2004: Elyemni *et al.*, 2019; Lo Presti *et al.*, 2005). Our work was based on the chemical variability of *P. Scoparius* essential oil as well as the antimicrobial and the anti-inflammatory activities depending on the extraction methods. To the best of our knowledge, this is the first study Dedicated to extractions using the microwave assisted hydrodistillation is reported here for the first time. In addition, no pharmacological studies on the anti-inflammatory activity of essential oils were previously conducted. The aim of our work is to check the variation of the chemical composition, antimicrobial and anti-inflammatory activities of the essential oils using three extraction methods (hydrodistillation, micro-wave and steam distillation).

## 2. Materials and methods

### 2.1. Samples

The aerial parts of *P. Scoparius* were collected from the Algerian Sahara (Taessa, Tamanrasset) in July of 2012. The harvested plants were washed and then allowed to dry in the shade in a dry and ventilated place. Global Positioning System (GPS) technology was used to record the location: latitude (23º05′64″ N), longitude (5º30′79,7″ E) and altitude (1748 m). Identification of plant was performed in the Botany laboratory of the National Forest Research Institute in Tamanrasset (NFRI).

### 2.2. Essential oils

#### *2.2.1. Hydro-distillation (HD)*

(200 g) of plant were placed in a flask. The flask was then filled with (4L) of water. The flask containing the plant and water was brought to a boil for 4hours using Clevenger-type apparatus (Council of Europe, 2010). The

essential oil-laden water vapors then passed through the refrigerant, condensed and fell into a separating funnel. The water and oil separated by density difference. All samples were collected and subsequently stored (sealed brown vial) at 4°C in the dark for further experimentation. The yield, based on the dry weight of the sample, was calculated.

***2.2.2. Microwave-assisted extraction (MAE)***

Microwave-assisted extraction was used at atmospheric pressure at (850 W, 2450 MHz) power for 50 minutes (Beoletto *et al.*, 2016), using a laboratory microwave for extracting the essential oil (M937, Samsung, United Kingdom). (100 g) of aerial part was placed in a (1L) flask with (100 ml) of water and connected to the Clevenger-type apparatus located outside the microwave. The essential oil was collected in the same way as mentioned in the HD process. The yield, based on dry weight of the sample, was calculated.

***2.2.3. Steam distillation (SD)***

The steam was passed through a (5L) flask containing (200 g) of plant for 4 h. The steam was then passed through a coiled tube where it was condensed. The distillate was collected. Finally, the essential oil obtained was separated through decantation using a separating funnel (Pushpangadan *et al.*, 2012). The essential oil was collected in the same way as mentioned in the HD process. The yield, based on dry weight of the sample, was calculated.

**2.3. Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS) analysis**

Analyses of the essential oils were carried out using a Hewlett-Packard 6800 GC equipped with an HP5-MS capillary column (30m x 0.32mm x 0.25 m) film thickness. The oven temperature was kept at 60°C for 8 min initially, then gradually increased to 250°C at 2°C/ min and kept again at 250°C for 20 min. The injector and detector temperature were 250 °C and 280 °C, respectively. The carrier gas was helium used at flow rate of 1.2 mL/min. 1µl of sample was injected in split mode at a ratio of 1:70, where the detection ionization energy was 70 eV. For GC-MS analysis, the same above-mentioned conditions for GC were applied. The mass scan range was 29-550 Uma. The percentage composition of the oils was expressed as peak area by integration from the total ion current. The percentage of each compound was computed using the normalization method from the GC peak area, calculated as mean value of three injections, without using correction factors. The identification of the compounds was based on the comparison of retention indices with those reported in the literature. Retention indices were calculated using the Van Den Dool and Kratz equation. The series of n-alkanes ($C_5$-$C_{28}$) were injected in the same conditions. The EI–mass spectra of essential oils were compared with those of mass spectra library (NIST and Wiley 7N library) and the literature (Adams, 2007).

**2.4. Pharmacological activities**

***2.4.1. Animals***

Strains of albino mice of both sexes, weighing (20-25g) were used for pharmacological studies. The animals were obtained from the Department of Pharmacotoxicology, SAIDAL Antibiotical-Medea, Algeria. The animals were housed in cages under standard laboratory conditions (12-hour light/dark cycle at 25°C). The animals were divided into groups of five and fasted for 12 hours before the experiment.

***2.4.2. Acute toxicity assay***

The acute toxicity test was performed using the Lorke method (Lorke, 1983). Mice were divided into several groups of five. (0.5 mL) of (2000 mg / kg) of essential oils were administered orally. The control group was treated in the same manner without administering essential oil. The mice were observed for 14 days. The mice that died in each group were counted for lethal dose (LD50) determination.

***2.4.3. Anti-inflammatory activity***

The anti-inflammatory activity was determined based on the carrageenan induced edema test (Levy, 1969). Groups of five mice each were treated orally with (0.5 mL) of

essential oils (50 mg/kg, 100 mg/kg and 150 mg/kg). After 30 minutes, (0.025 mL) of (1%) carrageenan solution (diluted with 0.9% physiological water) was injected into the right hind paw planter surface of the mice. Positive control received (50 mg/kg, 100 mg/kg and 150 mg/kg) of Diclofenac. After 3.30 hours, the animal suffers euthanasia and their right and left paws were removed and weighed with a precision scale. The percentage of edema was calculated according to the following equation:

% Edema = 100 × (Average weight of left paws - average weight of right paws) ÷ Average weight of left paws.

The percentage reduction of edema in the treated mice relative to the control was calculated according to the following equation:

% Reduction of edema= 100 × (% Edema control - % Edema test) ÷ % Edema control.

#### *2.4.4. Antimicrobial activity*

*2.4.4.1. Microbial strains*

Nine stains were used, including five Gram-positive bacteria: *Staphylococcus Aureus* ATCC 6538/P, *Staphylococcus Epidermidis* ATCC 12228, *Enterobacter Faecalis*, *Bacillus Subtilis* ATCC 6633 and *Sarcina Lutea*, two Gram-negative bacteria: *Escherichia Coli* ATCC 11105 and *Pseudomonas Aeruginosa* ATCC 27853 and two yeasts: *Candida Albicans* ATCC 10231 and *Saccharomyces Cerevisiae* ATCC 2601.

The microbial strains belonged to the American Type Culture Collection, except for *Enterobacter Faecalis*, and were supplied from Frantz Fanon Hospital in Blida, Algeria and Pastor Institution in Algiers, Algeria.

*2.4.4.2. Agar disk diffusion method*

The antimicrobial activity was determined using the disk diffusion method (National Committee for Clinical Laboratory Standards, 1997).The test was performed using the Soja Agar medium for bacterial germs and Sabouraud medium for fungal germs. The microbial suspensions were adjusted to $10^6$ CFU/ml (Colony Forming Units) in a sterile saline solution and were streaked over the surface of the plates using a sterile cotton swab in order to get a uniform microbial growth on the test plates. Sterile filter paper disks (6 mm in diameter) were then permeated with (10 uL) of essential oil. Plates were incubated at 37°C for 24h. Penicillin G, Oxalin and Amoxypen (200 mg/mL) were used as standard antibiotics. The antimicrobial activity was evaluated by measuring the diameter of inhibition in mm (diameter of the disc included). Assays were performed in triplicate.

### 2.5. Statistical analysis

Collected data are expressed as mean ± standard deviation. All tests were performed using IBM SPSS v 23. The comparison between chemical compositions was performed using a multivariate analysis of variance, sustained by a Tukey post hoc test, if significant. The study of biological activity was established by the ANOVA or Kruskal Wallis tests, sustained by Tukey or Mann Whitney U tests, if significant. Differences are considered as significant when ($p<0.05$), highly significant when ($p<0.01$) or strongly significant when ($p<0.001$).

## 3. Results and discussions

### 3.1. Yields and chemical composition of essential oils

The extraction of the aerial parts of *P. Scoparius* produced yellow essential oils ranging in yield from 0.20% to 0.53% (w/w) as a function of the extraction method (Table 1). The results showed that the extraction method had a significant influence on essential oil yields of *P. Scoparius* ($p <0.05$). The extraction by HD provided the highest yield (0.53%, 4h) compared to that obtained by SD (0.33 %, 4h), however the lowest yield was obtained using MAE (0.20 %, 0.5h). The results obtained agreed with those reported by several authors (0.25–0.99 %) (Vérité and Nacer, 2003; Lograda *et al.*, 2013; Hammoudi *et al.*, 2015; Ksouri *et al.*, 2017; Malti *et al*, 2018, Attia *et al.*, 2011). However higher oil yields were obtained from some different Algerian Saharan regions in

others studies (1.0-2.8%) (Gourine *et al.,* 2011; Lograda *et al.*, 2013).

**Table 1.** Variability in the chemical composition of *P. Scoparius* essential oil obtained by different methods

| No. | Compounds[a] | RI[b] | RI[c] | HD | MAE | SD |
|---|---|---|---|---|---|---|
| **1** | *α*-Thujene | 914 | 930 | 1.94 | - | 0.49 |
| **2** | *α*-Pinene | 925 | 939 | 10.84 | 0.48 | 3.28 |
| **3** | Camphene | 937 | 953 | 0.16 | - | tr |
| **4** | Sabinene | 967 | 976 | 0,88 | tr | 0.26 |
| **5** | *β*-Pinene | 971 | 979 | 2.63 | 0.27 | 1.19 |
| **6** | *β*-Myrcene | 989 | 990 | 0.38 | - | 0.14 |
| **7** | *I*-Phellandrene | 1003 | 1005 | 0.39 | tr | 0.18 |
| **8** | *Δ*-3-Carene | 1009 | 1011 | 1.08 | tr | 0.80 |
| **9** | *α*-Terpinene | 1015 | 1017 | 0.28 | tr | 0.25 |
| **10** | *p*-Cymene | 1025 | 1024 | 4.89 | 1.57 | 4.02 |
| **11** | Limonene | 1028 | 1029 | 3.16 | 0.77 | 1.73 |
| **12** | *β*-Ocimene*Z* | 1039 | 1040 | 2.70 | 0.32 | 1.00 |
| **13** | *β*-Ocimene*E* | 1047 | 1050 | 0.27 | - | tr |
| **14** | *γ*- Terpinene | 1057 | 1059 | 0.56 | 0.18 | 0.60 |
| **15** | *Cis*sabinene hydrate | 1064 | 1068 | 0.11 | tr | tr |
| **16** | *α*-Terpinolene | 1085 | 1088 | 0.24 | 0.19 | 0.38 |
| **17** | *Trans*sabinene hydrate | 1095 | 1097 | 0.22 | - | 0.13 |
| **18** | *β*-Thujone | 1113 | 1114 | 0.16 | - | 0.30 |
| **19** | *α*-Campholenal | 1117 | 1125 | - | 0.65 | - |
| **20** | Terpinene-1-ol | 1118 | - | 0.15 | - | - |
| **21** | *α*-Comphoaldehyde | 1123 | - | 0.37 | - | 0.53 |
| **22** | Pinocarveol (trans) | 1135 | 1139 | 0.69 | 0.92 | 0.57 |
| **23** | *Cis* Verbenol | 1138 | 1140 | 0.11 | 0.49 | - |
| **24** | *Trans* Verbenol | 1142 | 1144 | - | - | 0.27 |
| **25** | Penthylbenzene | 1154 | 1156 | 0.14 | - | 0.27 |
| **26** | Pinocarvone | 1160 | 1162 | 0.55 | 0.52 | 0.30 |
| **27** | 4-Terpineol | 1176 | 1177 | 1.04 | 1.10 | 1.36 |
| **28** | Crypton | 1179 | - | - | 0.22 | 0.26 |
| **29** | Cymen-8-ol-para | 1183 | 1183 | 0.30 | 0.37 | 0.44 |
| **30** | *α*-Terpineol | 1189 | 1189 | 0.18 | - | 0.29 |
| **31** | (-)Myrtenal | 1194 | 1193 | 0.44 | 0.37 | 0.24 |
| **32** | Myrtenol | 1200 | 1200 | 0.48 | 0.36 | 0.25 |
| **33** | *α*-Phellandrene epoxide | 1204 | - | - | 0.67 | 0.70 |
| **34** | *I*-Verbenone | 1206 | 1205 | 0.21 | 0.32 | 0.34 |
| **35** | *Trans*Carveol | 1218 | 1216 | 0.30 | 0.56 | 0.41 |
| **36** | Propanal,2-methyl-3-phenyl | 1238 | - | - | - | 0.41 |
| **37** | *I*-Carvone | 1241 | 1242 | 0.13 | 0.24 | 0.15 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **38** | Carvotanacetone | 1245 | 1247 | tr | 0.14 | 0.12 |
| **39** | Phellandral | 1273 | 1271 | 0.10 | - | 0.20 |
| **40** | Safranal | 1280 | - | - | 0.68 | - |
| **41** | *p*-Cymen-7-ol | 1287 | 1290 | tr | 0.18 | 0.22 |
| **42** | Thymol | 1289 | 1290 | - | - | 0.15 |
| **43** | Carvacrol | 1300 | 1299 | 0.30 | 0.61 | 0.73 |
| **44** | *α*-Copaene | 1370 | 1376 | 0.11 | 0.18 | 0.22 |
| **45** | Methyleugenol | 1400 | 1401 | 0.25 | 0.61 | 0.37 |
| **46** | *β*-Selinene | 1481 | 1485 | 0.21 | 0.11 | 0.22 |
| **47** | Myristicin | 1523 | 1520 | 4.21 | 9.35 | 7.15 |
| **48** | *α*-Calacorene | 1533 | 1545 | tr | 0.11 | 0.18 |
| **49** | *β*-Calacorene | 1549 | - | 0.39 | - | - |
| **50** | Élémicine | 1556 | 1554 | 0.14 | 0.33 | 0.28 |
| **51** | 1,5-epoxysalvial-4 (14) ene | 1560 | - | 0.62 | 0.60 | 0.66 |
| **52** | Spathulenol | 1575 | 1576 | 4.27 | 5.23 | 4.23 |
| **53** | Salvia-4 (14)-en-1-one | 1586 | - | 0.44 | 0.76 | 0.71 |
| **54** | Caryophyllene oxide | 1600 | 1581 | 0.57 | - | 0.24 |
| **55** | Dillapiol | 1612 | 1622 | 16.38 | 37.21 | 31.95 |
| **56** | Isospathulenol | 1634 | - | 0.52 | 0.18 | 0.22 |
| **57** | *β*-eudesmol | 1645 | 1649 | 9.19 | 7.10 | 7.70 |
| **58** | Butylidenephtalide*Z* | 1668 | 1672 | 7.15 | 1.23 | 6.65 |
| **59** | Butylidenephtalide*E* | 1700 | 1718 | 1.86 | 2.72 | 2.20 |
| **60** | 3-N-Butyl phtalide | 1724 | 1739 | 4.46 | 3.50 | 2.86 |
| **61** | Butylidenedihydro-phtalide | 1777 | - | 0.33 | - | 0.14 |
| **62** | Hexahydrofarnesyl acetone | 1848 | - | - | 0.32 | - |
| **63** | Acidepalmitique | 1947 | - | 0.21 | 0.73 | tr |
| **Yield** | | | | 0.53 | 0.20 | 0.33 |
| Monoterpene Hydrocarbons | | | | 30.16 | 3.59 | 13.94 |
| Oxygenated Monoterpenes | | | | 6.22 | 8.59 | 9.02 |
| Sesquiterpene Hydrocarbons | | | | 5.17 | 10.36 | 8.14 |
| Oxygenated Sesquiterpenes | | | | 32.13 | 51.41 | 45.99 |
| Phtalides | | | | 13.8 | 7.45 | 11.85 |
| Others | | | | 0.21 | 1.05 | tr |
| **Total** | | | | 87.69 | 82.45 | 88.94 |

[a] Compounds listed according to their elution order on apolar HP5MS ™ capillary,[b]Retention Indices on apolar column (HP5MS), [c] Retention indices of literature on apolar column reported by Adams (2007), tr: trace (<0.1%), −: Not detected, all components were identified by comparison of their retention indices with those of published data (Adams, 2007) and mass spectra with literature data, the MS library (NIST and Wiley 7N library)

GC and GC-MS analyses resulted in the identification of sixty-three compounds representing (82.45-88.94%) of the total composition of the essential oils. As shown in (Table 1), a noteworthy qualitative similarity was observed, but with differences in the abundance of major compounds. The oxygenated sesquiterpenes were found to be the

main chemical group in all samples (32.13-51.41%). The second most frequently identified chemical class in the hydrodistillated sample was monoterpene hydrocarbons (30.16%). This fraction was present in much lower amounts in the samples obtained by SD and MAE (13.94 and 3.59%, respectively). The oxygenated monoterpene compounds were present in lower amounts in all samples (<10%). Among the three extractive processes, an important observation revealed that MAE isolated the highest relative amount of oxygenated sesquiterpenes (51.41%), as compared to SD with (45.99%) and HD with (32.13%). This result, which indicates that oils isolated by Microwave-assisted hydrodistillation are characterized by a higher content of oxygenated compounds, was previously reported on essential oils of three different species (Ocimum *basilicum L.*, *Mentha crispa L.* and *Thymus vulgaris L.*) (Lucchesi *et al.*, 2004). The same finding was obtained on *Rosmarinus officinalis L.* essential oils by (Elyemni *et al.,* 2019; Karakayat *et al.,* 2014; Okoh *et al.,* 2010; Moradi *et al.,* 2018).This may be due to an increase in hydrothermal effects in HD, compared to MAE which uses a lower quantity of water, that is rapidly heated [20, 30].Conversely, Lo Presti, M.et al. reported in their study on the same species (*Rosmarinus officinalis L.*) that the essential oils produced by MAE and HD were characterized by very similar chemical profiles (Lo Presti et al., 2005). Alternatively, a different result was previously published, where the amount of the oxygenated compounds was higher in the hydrodistillated essential oil compared with the oil isolated by microwave distillation from dried *Cuminum cyminum L.*, and *Zanthoxylum bungeanum Maxim* (Wang *et al.,* 2006). This contradiction in results is probably due to the fact that the amount of oxygenated compounds in essential oil is not dependent only on extraction methods, but also on many other factors including: species used, plant maturity, plant part used and harvest site (Mohammedi *et al.*, 2015; Mohammedi *et al.*, 2019).

The major compounds identified in the three essential oils were Dillapiol, β-eudesmol, Myristicin, α-Pinene and Butylidene phtalide Z, but with significant differences in their proportions depending on the extraction method used. Dillapiol, the major compound in all samples, was present at (37.21%) and (31.95%), respectively, for MAE and SD, but only at (16.38%) for HD. In the same way, Myristicin, the second compound in MAE oil (9.35%), amounted to (7.15%) and (4.21%) in the SD and HD samples, respectively. The hydrodistillated oil contained the highest amount of α-Pinene (10.84%), which is present in much lower amount in the other oils obtained by SD and MAE (3.28% and 0.48%, respectively). Both samples extracted by HD and SD contained important amounts of Butylidene phtalide Z (7.15% and 6.65%, respectively) and p-cymene (4.89% and 4.02%, respectively).These compounds account for only (1.23-1.57%) in the oil obtained by MAE. Other major compounds were determined with equivalent amounts in the oils extracted by HD, SD and MAE such as β-eudesmol (9.19%, 7.70% and 7.10%, respectively), Spathulenol (4.27%, 4.23% and 5.23%, respectively) and 3-N-Butyl phtalide (4.46%, 2.86% and 3.50%, respectively). To the best of our knowledge, 3-N-Butyl phtalide has never been identified in *P. Scoparius* essential oils.

The multivariate analysis of variance showed a strong significant effect of methods on major essential oil compounds. ($p<0.001$).Extraction method variation indicates a significant effect on: Dillapiol ($p<0.001$); α-Pinene ($p<0.001$); Butylidene phtalide Z ($p<0.001$); β-Ocimene Z ($p<0.001$); β-Pinene ($p<0.001$); α-Thujene ($p<0.001$); Myristicin ($p<0.001$); Limonene ($p<0.001$); p-Cymene ($p<0.001$); 3-N-Butyl phtalide ($p<0.001$); β-eudesmol ($p<0.001$); Spathulenol ($p<0.001$); Δ-3-carene ($p<0.001$); Butylidene phtalide E ($p<0.001$); 4-terpineol ($p=0.002$). The mean comparison between major compounds was assessed to choose the best extraction method for each Substance. Results show a considerable difference between methods for most

compounds, except for 4-terpineol, for which results indicate no difference in SD vs MAE (p=0.074), as well as Spathulenol, for which results indicate no difference in SD vs HD (p=0.887).

**Table 2.** Major compounds of *P. Scoparius* essential oils from different origins previously reported.

| Country / Region | | Used part | Main constituents (%) | | | | | | Ref |
|---|---|---|---|---|---|---|---|---|---|
| | | | α-pinene | Sabinene | Limonene | Myristicin | Dillapiol | Other major constituents | |
| Algeria | Oum El Bouaghi | S | 34 | - | - | - | - | Apiol (15.0) | Vernin *et al.*, 1999 |
| | | d | 11 | - | - | - | - | Apiol (52.8); Bornyl acetate (21%). | |
| | Ghardaïa | S | 6.8 | - | 9.8 | 7.2 | - | Germacrene D (12.7); *α*-Phellandrene (7.1); Methyl eugenol (5.9). | Vérité and Nacer, 2003 |
| | | d | 8.2 | - | 11.2 | 11.1 | - | Caryophyllene oxide (12.2); *p*-Cymene (7.5); Thymol (5.9). | |
| | M'sila | F | 17.4 | 7.5 | - | 24.1 | - | *α*-Phellandrene (15.6). | Smaili *et al.*, 2011 |
| | Ghardaïa | P | 4.4-11.2 | - | 32.7-66.5 | ≤ 31.1 | ≤ 23.0 | *α*-Phellandrene (≤ 6.4); Germacrene D (≤ 6.3). | Gourine *et al.*, 2011 |
| | Djelfa | P | 23.7-27.0 | - | 1.0-7.8 | ≤ 18.2 | ≤ 47.3 | Bornyl acetate (≤ 9.6); *p*-Cymene (2-6.7); *β*-Pinene (≤ 5.3). | |
| | Laghouat | P | 35.1-35.8 | - | 7.0-30.0 | - | 9.9-25.7 | Bornyl acetate (3.0-9.5); *β*-Pinene (5.2). | |
| | M'sila | P | 16.4 | 14.8 | - | - | - | Caryophyllene oxide (9.7); *α*-Farnesene (7.7); *α*-Terpinene (5.8). | Lograda *et al.*, 2013 |
| | Batna | P | 23.3 | 18.6 | - | - | - | *α*-Terpinene (7.7); *β*-Ocimene-E (7.6). | |
| | Biskra | P | 8.3-13.4 | 18.9-24.8 | - | 7.6 | 6.6-16.8 | - | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tamenra -set | P | 12.1 | - | - | - | - | Bornyl acetate (32); Epi-Bicycle sesquiphellandrene (8.4); Eremophilene (8.2); γ-Cadinène (6.3). | Hammoudi *et al.*, 2015 |
| | Ghardaïa | P | - | - | - | 12.1 | - | 7-Methoxy-3-methyl-1-H-isichromen-1-one (10.6); Methyl propene (9.9). | Belkacemi *et al.*, 2015 |
| | Souk Ahras | P | 16.3-26.5 | 23.6-34.4 | - | - | - | *p*-Cymene (8.6-10.1); Terpinen-4-ol (4.1 - 9.7); *α*-Thujene (5.3-5.7); *β*-Pinene (≤ 5.5). | Chikhoune *et al.*, 2016 |
| | Tamenra s -set | P | - | - | 46.9 | - | - | 1.8-Cineol (7.6). | Ksouri *et al.*, 2017 |
| | Batna | P | ≤ 11.2 | ≤ 10.5 | ≤ 22.4 | ≤ 19.4 | ≤ 16.8 | 6-Methoxyelemicine (≤ 58.2). | Malti *et al.*, 2018 |
| | Biskra | P | 7.2-11.2 | 19.9-28.0 | - | ≤ 20.1 | ≤ 18 | Elemicine (≤ 29.1). | |
| | Bechar | P | - | 32.4 | 19.1 | - | - | 6-Methoxyelemicine (27.4). | |
| | Ghardaïa | P | 2.8-17.1 | ≤ 31.3 | 9.2-26.7 | ≤ 13.6 | - | 6-Methoxyelemicine (≤ 29.4); *α*-Phellandrene (≤ 15.4); *β*-Phellandrene (≤ 7.9); *β*-Pinene (≤ 7.1). | |
| **Tunisia** | | P | 32.0 | 17.2 | - | - | - | Δ-3-Carene (16.9); *α*-Thujene (13.7); Ocimene (9.8). | Attia *et al.*, 2011 |

P: Aerial parts; S: Stems; F: Flowers; d: seeds; n.r: not reported.

Previous research on the chemical composition of *P. Scoparius* essential oils obtained by HD and SD from different origins is summarized in (Table 2). It is noted that no study on the essential oil of *P. Scoparius* as a function of extraction method has been performed to-date. As mentioned above, a significant qualitative and quantitative

difference in the chemical compositions was observed. As shown in (Table 2), most samples, whether from Tunisia or from different Algerian regions (M'sila, Batna, Beskra and Souk Ahras) were characterized by a high amount α-Pinene (8.3-35.8%) and Sabinene (14.8-34.4 %) (Lograda *et al.*, 2013; Chikhoune *et al.*, 2016; Attia *et al.*, 2011). Otherwise, essential oils obtained from seeds and stems essential oils from Oum El Bouaghi were dominated by apiol (52.8% and 15%, respectively) and α-pinene (11% and 34%, respectively). Bornyl acetate was present with large amount only in the seeds oil (21%) (Vernin *et al.*, 1999). Limonene (32.7-66.5%), Myristicin (up to 31.1%), dillapiol (up to 23%) and α-pinene (4.4-11.2%) were the main compounds of the aerial part essential oil from Ghardaia (Gourine *et al.,* 2011), while, Germacrene D (12.7%) was the major compound of the stems oil and Caryophyllene oxide (12.2%) the major compound of the seeds from same region (Ghardaïa). These two samples were also characterized by similar amounts of limonene, myristicin and α-pinene (6.8-9.8% and 8.2-11.2%, respectively for stems and seeds oils) (Vérité and Nacer, 2003). Samples from Djelfa and Laghouat showed similar chemical profiles, dominated by α-pinene (23.7-35.8%), dillapiol (9.9-47.3%), Limonene (up to 30.0%), bornyl acetate (3.0-9.6%) and β-Pinene (up to 5.3%). However, myristicin was present in high amounts only in the oil obtained from Djelfa (up to 18.2%) (Gourine *et al.,* 2011). Myristicin 24.1 % and α-pinene (14.4 %) were also the primary compounds in the flours oil from M'sila, followed by α-Phellandrene (15.6 %) (Smaili *et al.*, 2011). The essential oils of *P. Scoparius*, isolated from Batna, Bechar and Ghardaïa, contained the same dominant compounds: 6-Methoxyelemicine (0.0–59.6%), sabinene (0.8–55.6%), limonene (0.3–44.0%), myristicin (0.0–32.4%) and α-pinene (0.7 –31.0%). Dillapiol was found as a principal compound only in the oil from Batna (up to 16.8%) (Malti *et al.*, 2018). 6-methoxyelemicine was identified neither in our work, nor in other reports. In the same study, the samples coming from Biskra were dominated by sabinene (19.9-28.0%), elemicine (up to 29.1%) and myristicin (up to 20.1%), while dillapiol and α-pinene were also present with appreciable percentages (1.4-18% and 7.2-11.2%, respectively) (Malti *et al.*, 2018). Limonene (46.9%) was the predominant compound in the sample from Tamenrasset, followed by 1.8-Cineol (7.6) (Ksouri *et al.*, 2017). In contrast, α-pinene (12.1%), Epi-Bicyclosesqui phellandrene (8.4%), Eremophilene (8.2%) and γ-Cadinene (6.3%) were the main components of the hydrodistillated oil from the same region (Tamenrasset) (Hammoudi *et al.*, 2015). Furthermore, 7-Methoxy-3-methyl-1-H-isichromen-1-one and methyl propene were identified, along with myristicin, in appreciable amounts only in the oil obtained by SD from Ghardaïa (10.6%, 9.9% and 12.1%, respectively) (Chikhoune *et al*., 2016).

### 3.2. Acute Toxicity Test

The oral administration of *P. Scoparius* essential oils in doses of 2000 mg/kg (limited dose) did not cause any mortality in treated mice for 14 days following the oral administration. According to Hodge and Sterner scale (Hodge, 1943), *P. Scoparius* essential oil can be classified as non-toxic.

### 3.3. Anti-inflammatory activity

In the present investigation, the anti-inflammatory activity of Diclofenac and *P. Scoparius* essential oils extracted by HD, MAE and SD was determined based on the carrageenan induced edema test. The results obtained are expressed in triplicate in (Table 3).

Our results showed that all the studied samples exhibited anti-inflammatory activity in a dose-dependent manner. In fact, the concentration had a significant effect on the extraction methods ($p<0.05$) with the exception of MAE ($p = 0.308$). The groups treated with essential oils at a dose of 50 mg / kg showed the lowest inhibition percentages of carrageenan-induced edema (25.31 ± 0.85-40.64 ± 2.36%) compared to the groups treated with doses of 100 mg / kg (27.75 ± 0.53-44.36 ± 2.30 %).

**Table 3.** Effects of *P. Scoparius* essential oils and Diclofenac on carrageenan-induced hind paw edema in mice

| Doses (mg/kg) | Inhibition (%) | | | |
|---|---|---|---|---|
| | **HD** | **MAE** | **SD** | **Diclofenac** |
| 50 | $25.31\pm0.85^{c, C}$ | $40.64\pm2.36^{a, A}$ | $31.37\pm3.38^{b, B}$ | $42.69\pm3.04^{a, C}$ |
| 100 | $27.75\pm0.53^{b, B}$ | $44.36\pm2.30^{a, A}$ | $33.76\pm4.33^{b, B}$ | $63.32\pm1.83^{a, B}$ |
| 150 | $55.68\pm1.30^{b, A}$ | $48.15\pm2.82^{c, A}$ | $51.09\pm3.68^{bc, A}$ | $69.01\pm1.00^{a, A}$ |

Values are averages ± standard deviation of triplicate analysis.
Data in the same column having different capital letters are significantly different ($P < 0.05$) among different concentrations.
Data in the same row having different lower-case letters are significantly different ($P < 0.05$) among different essential oil extraction method.
Results are ranked in ascending order; a>b>c; A>B>C.

The maximum activity was observed at 150 mg / kg for all samples (48.15 ± 2.82-55.68 ± 1.30%). The percentage of inhibition of Diclofenac (50, 100 and 150 mg / kg), taken as a standard drug, also increased from (42.69 ± 3.04%) to (69.01 ± 1.00%), with increasing dose. At a dose of 50 mg / kg, HD was less effective than the sample obtained by SD (25.31 ± 0.85 vs 31.37 ± 3.38%). However, using MAE, the essential oil showed the highest inhibition percentage (40.64 ± 2.36%). The last value was comparable to that obtained with Diclofenac (42.69 ± 3.04%) and had no significant difference with the standard drug ($p = 0.743$). The same order was observed using samples at a dose of 100 mg / kg (27.75 ± 0.53, 33.76 ± 4.33% and 44.36 ± 2.30%, respectively for HD, SD and MAE). The results showed that the anti-inflammatory activity of HD vs SD at the dose of 100 mg / kg has no significant difference ($p = 0.667$). The same is true for MEA and Diclofenac ($p = 0.099$). In contrast, at a dose of 150 mg / kg, the maximum activity was obtained in HD (55.68 ± 1.30%) and presented no significantly difference from SD ($p=0.147$).

Based on these results, it can be asserted that *P. Scoparius* essential oils have anti-inflammatory activity, with significant difference depending on the method of extraction used. This important anti-inflammatory activity of *P. Scoparius* essential oils can be attributed to their content of dillapiol, β-eudesmol, myristicin, Butylidene phtalide Z and α-Pinene. These results validate the use of *P. Scoparius* as an anti-inflammatory in traditional Algerian medicine.

### 3.4. Antibacterial assays

The antimicrobial activity of essential oils was determined against seven bacteria strains and two yeasts using the disk diffusion method. This activity is estimated based on the diameter of inhibition zones. Results are presented in (Table 4) for different extraction methods.

Our data showed that *P. Scoparius* essential oils generally possessed a weak to moderate activity against yeasts (inhibition zone from 7.1±0.1 to 13.6±0.4 mm) as well as gram-positive bacteria (inhibition zone from 8.2±0.1 to 21.8±0.3 mm).

*S. lutea* was the most affected by *P. Scoparius* essential oils; the oil extracted by HD showed the largest zone of inhibition (inhibition zone = 21.8±0.3 mm) as compared to those obtained by MAE (inhibition zone = 19.8±0.7 mm) and by SD (inhibition zone = 14.7±0.6 mm). Similarly, the tested oils exhibited weak activity against *S. aureus* (inhibition zone: 8.2±0.1-11.0±0.1 mm) and *B. subtilus* (inhibition zone: 9.1±0.6-11.2±0.2 mm), while *S. epidermidis* was more affected by the oils obtained by HD and SD (inhibition zone ≈ 12.7 mm). Dillapiol had no effect against *S. aureus* (Ferreira *et al.,* 2016). In another study;

Dillapiol had no effect against gram-negative bacteria and weak effect against gram-positive bacteria (Eftekhar *et al.,* 2014). This limited effect of *P. Scoparius* essential oils against *S. aureus* could be due to their high content of dillapiol.

These results were previously reported by Ksouri, A. *et al.* on *B. bastilus* (inhibition zone = 8.3±1.1 mm) while *S. aureus* was more resistant to *P. scoparius* essential oil (inhibition zone = 20.0±3.0 mm) (Ksouri *et al*., 2017). Kiram, A. *et al.* who worked on *P. scoparius* from Biskra (Southeast Algeria) reported stronger oil activity against the same bacterial strain, *S. aureus* [Kiram *et al.,* 2013).

**Table 4.** Antimicrobial activity of *P. Scoparius* of essential oils used three different methods extractions.

| Microorganisms | Inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Essential oil | | | Standard antibiotics | | |
| | HD | MAE | SD | Penicillin G | Oxalin | Amoxypen |
| **Gram- positive bacteria** | | | | | | |
| *S. aureus* | $8.2\pm0.1^{b,F}$ | $8.2\pm0.1^{b,D}$ | $11.1\pm0.1^{a,B}$ | 40 | 40.6 | 38 |
| *S. epidermidis* | $12.4\pm0.3^{a,C}$ | $10.4\pm0.2^{a,C}$ | $12.1\pm0.6^{a,AB}$ | 14.6 | 21.8 | 17.6 |
| *S. lutea* | $21.8\pm0.3^{a,A}$ | $19.8\pm0.7^{a,A}$ | $14.7\pm0.6^{b,A}$ | >45 | >45 | >45 |
| *B. subtilus* | $11.2\pm0.2^{a,D}$ | $9.1\pm0.6^{b,D}$ | $9.5\pm0.1^{b,C}$ | 35 | 40 | 34 |
| **Gram-negative bacteria** | | | | | | |
| *E. faecalis* | - | - | - | 12 | 25 | 26 |
| *E. coli* | - | - | - | 31 | 16.9 | 40 |
| *P. aeruginosa* | - | - | - | - | 17 | 19 |
| **Yeast** | | | | | | |
| *C. albicans* | $11.5\pm0.3^{b,D}$ | $11.3\pm0.2^{b,B}$ | $13.4\pm0.2^{a,A}$ | - | - | - |
| *S. cerevisiae* | $13.6\pm0.4^{a,C}$ | $7.1\pm0.1^{b,E}$ | $12.0\pm0.1^{a,A}$ | - | - | - |

Values are averages ± standard deviation of triplicate analysis, zone of inhibition in mm ± standard deviation beyond well diameter (6 mm), data in the same column having different capital letters are significantly different ($P < 0.05$) among different microorganisms, data in the same row having different lower case letters are significantly different ($P < 0.05$) among different essential oil extraction method, results are ranked in ascending order; a>b; A>B>C>D>E>F, -: not action

Alternatively, *P. scoparius* essential oils showed no activity (inhibition zone = 0 mm) against gram-negative bacteria *E. faecalis*, *E. coli* and *P. aeruginosa*. Unlike gram-positive bacteria, it is known that gram-negative bacteria have a very high resistance to essential oils due to their wall made up of a second external barrier (Okoh *et al*., 2010; Dhouioui *et al*., 2016). The same results were obtained by other authors who reported that *P. Scoparius* essential oils generally showed limited activity against *P. aeruginosa*, *E coli* and *E. faecalis* (Ksouri *et al.,* 2017; Ferreira *et al.,* 2017).

Our findings displayed a strong significant difference against *S. aureus* and *S. cerevisiae* between MEA and SD ($p<0.001$), and no significant difference between HD and MEA against strains ($p>0.05$), except for *B. subtilus* and *S. cerevisiae*. The comparison of HD vs SD showed that the extraction method influenced the antimicrobial activity except for *S. epidermidis* and *S. cerevisiae* ($p <0.05$).This could be due to the high content of hydrocarbon monoterpene since the antimicrobial activity of the essential oil could be linked to the presence of α-pinene (Bourkhiss *et al.,* 2007; Abi-ayada *et al.,* 2011; Amarti *et al*., 2011)); although minor molecules can also produce antibacterial activity, contributing to the activity of essential oils (Okoh *et al.,* 2010).

## 4. Conclusions

The effects of extraction methods on *P. Scoparius* essential oil compositions and their anti-inflammatory and antimicrobial activities are investigated here for the first time. The chemical compositions of the essential oils obtained are qualitatively similar, but with significant differences in the abundance of major compounds. *P. Scoparius* essential oils are non-toxic and exhibit important anti-inflammatory activity and weak antibacterial activity. The extraction method significantly affected the anti-inflammatory and antibacterial activities. It is very important to continue this research and find a correlation between the extraction techniques and their effects on the chemical composition and biological activities.

## 5. References

Abdellah, H., Sahki, R. E. (2004). Eds. Le Hoggar: Promenade botanique, Herbaceous species.

Abi-Ayada, F. Z., Abi Ayad, M., Lazzouni, H. A., Rebiahi, S., Bessiere. A. (2011). Antibacterial Activity of Essential oil extracted from leaves of *Tetraclinis articulata* (Vahl) Master from Algeria flora, *Journal of Microbiology and Biotechnology Research*, 1, 1-6.

Adams, R.P. (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. (4th ed.). Allured Pub Corp.

Amarti, F., Satrani, B., Ghanmi, M. Farah, A., Aafi, A., Aarab, L., El Ajjouri, M., Chaouch, A. (2010). Composition chimique et activité antimicrobienne des huiles essentielles de Thymus algeriensis Boiss. & Reut. et *Thymus ciliatus* (Desf.) Benth. du Maroc. Biotechnology, Agronomy, Society and Environment / Biotechnology, Agronomy, *Society and Environment*, 14(1), 141-148.

Attia, S., Grissa, K.L., Lognay, G., Heuskin, S., Mailleux, A.C., Hance, T. (2011). Chemical composition and acaricidal properties of *Deverra Scoparia* essential oil (Araliales: Apiaceae) and blends of its major constituents against Tetranychus urticae (Acari: Tetranychidae). *Journal of Economic entomology*, 104(4), 1220-1228.

Belkacemi, D., Kalla, A., Rouabhi R., Amara, D. (2016). Effect of *Pituranthos Scoparius* essential oils on reducing metganogenesis in cheep: in vitro Study. *Environmental Science and Indian Journal*, 12(3), 118-126.

Benchelah, A. C., Bouziane, H., Maka, M., Ouahes, C. (2000). Fleurs du Sahara : Voyage ethnobotanique avec les Touaregs du Tassili. 2nd eds. Ibis Press, 105-106.

Beoletto, V. G., De las Mercedes Oliva, M., Marioli, J. M., Carezzano, M. E., Demo, M. S. (2016). Antimicrobial Natural Products Against Bacterial Biofilms. Mechanisms and New Antimicrobial Approaches, *Antibiotic resistance*, Chapitre 14, 291–307.

Boukef, M. K. (1986). Plants in traditional Tunisian medicine. *Cultural and technical cooperation agency*, 1986, 228-230.

Bourkhiss, M., Ouhssine, M., Hnach, M., Satrani, B., Farah, A. (2007). Composition chimique et bioactivité de l'huile essentielle des rameaux de *Tetraclinis articulata*. *Bulletin of the Bordeaux Pharmacy Society*, 146, 75-84.

Chikhoune, A., Pavleca, J. D., Shashkov, M., Berroua, Z., Chebbi, K., Bougherra, H., Zeroual, B., Aliane, K., Gagaoua, M., Boudjellal, A., Vovk, I., Križman, M. (2016). Antioxidant effect induced by the essential oil of *Pituranthos Scoparius* in a formulation of a whey spread emulsion. *Journal of Food Processing and Preservation*, 41(5), 1-12

Council of Europe, Pharmacopée Européenne, (2010). 7th Eds. Directorate for the Quality of Medicines & HealthCare of the Council of Europe, Strasbourg, France.

Dhouioui, M., Boulila, A., Chaabane, H., Saïd Zina, M., Casabianca, H. (2016). Seasonal changes in essential oil composition of *Aristolochia longa L. ssppaucinervis Batt*. (Aristolochiaceae) roots and its antimicrobial activity. *Industrial Crops and Products,* 83, 301–306.

Eftekhar, F., Yousefzadi, M., Azizian, D., Sonboli,A., Salehi, P. (2005). Essential Oil

Composition and Antimicrobial Activity of *Diplotaenia damavandica. Zeitschrift für Naturforschung C*, 60(11-12), 821-825.

Elyemni, M., Louaste, B., Nechad, I., Elkamli, T., Bouia, A., Taleb, M., Chaouch, M., Eloutassi, N. (2019). Extraction of Essential Oils of *Rosmarinus officinalis* L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. *The Scientific World Journal*, 1-6.

Ferreira, R. G., C. Monteiro M. C., da Silva, J. K. R., Maia J. G. S. (2016). Antifungal Action of the Dillapiole-rich Oil of Piper aduncum against Dermatomycoses Caused by Filamentous Fungi. *Journal of Advances in Medicine and Medical Research,* 15(12), 1-10.

Gourine, N., Merrad, B., Yousfi, M., Stocker, P., Gaydou, E.M. (2011). Chemical composition of the essential oil of *Pituranthos Scoparius*. *Natural Product Communications*, 6(8), 1151-1154.

Hammiche, V., Maiza K. (2006). Traditional medicine in Central Sahara: pharmacopoeia of Tassili N'ajjer. *Journal of Ethnopharmacology*, 105(3), 358-367.

Hammoudi, R., Dehak, K., Hadj Mahammed, M., Didi Ouldelhadj, M. (2015) Composition chimique et activité antioxydante des huiles essentielles de *Deverra Scoparia* Coss. & Dur. (Apiaceae). *Lebanese Science Journal*, 16(2), 27-36.

Hodge, H.C., Sterner, J.H. (1943). Determination of substance acute toxicity by LD50. *American Industrial Hygiene Association Journal*, 9, 93-96.

Karakaya, S., Nehir El, S., Karagozlu, N., Sahin, S., Sumnu, G., Bayramoglu, B. (2014). Microwave-assisted hydrodistillation of essential oil from rosemary. *Journal of Food Science and Technology*, 51(6), 1056-1065.

Kiram, A., Ramdani, M., Zeraid, A. (2013). Etude Phytochimique et de L'activité Antimicrobienne des Huiles Essentielles de Pituranthos Scoparius de la Region de Biskra (Sud-Est Algérien). *Tunisian Journal of Medecinal Plants and Natural Products*, 10, 1-6.

Ksouri, A., Dob, T., Belkebir, A., Dahmane, D., Nouasri, A. (2017). Volatile compounds and biological activities of aerial parts of *Pituranthos Scoparius* (Coss and Dur) Schinz (Apiaceae) from Hoggar, southern Algeria. *Tropical Journal of Pharmaceutical Research*, 16(1), 51-58.

Levy, L. (1969). Carrageenan paw edema in the mouse, *Life Sciences*, 8(1), 601-606.

Lograda, T., Ramdani, M., Kiram, A., Chalard, P., Figueredo, G. (2013). Variation of essential oils composition of *Pituranthos Scoparius* in Algeria. *Global journal of research on medicinal plants and indigenous medicine*, 2(1), 1-9.

Lo Presti, M., Ragusa, S., Trozzi, A., Dugo, P., Visinoni, F., Fazio, A., Dugo, G., Mondello, L. (2005). A comparison between different techniques for the isolation of rosemary essential oil. *Journal of Separation Science*. 28(3), 273-280.

Lorke, D.A. (1983). New approach to practical acute toxicity, *Archives of Toxicology*, (pp. 53).

Lucchesi, M. E., Chemat, F., Smadja, J. (2004). Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. *Journal of Chromatography*, 1043(2), 323–327.

Malti, C., Boussaïd, M., Belyagoubi, L., Paoli, M., Gibernau, M., Tomi, F., AtikBekkara, F., Bekhechi, C. (2018). Chemical Variability of the Essential Oil O f *Pituranthos Scoparius* from Algeria, *Chemistry & Biodiversity*, 15(7).

Mohammedi, H., Mecherara-Idjeri, S., Foudil-Cherif, Y., Hassani, H. (2015). Chemical composition and antioxidant activity of essential oils from Algerian *daucus carota* L. subsp. *carota* aerial parts. *Journal of Essential Oil-Bearing Plants*, 18 (4), 873-883.

Mohammedi, H., Mecherara-Idjeri, S., Hassani, H. (2016). Variability in essential oil composition, antioxidant and antimicrobial activities of *Ruta montana* L. collected from different geographical regions in Algeria.

*Journal of essential oil research*, 32 (1), 88-101.

Morad, S., Fazlali, A., Hamedi. A. (2018). Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation. *Avicenna Journal of Medical Biotechnology*, 10(1), 22-28.

National Committee for Clinical Laboratory Standards. (1997). Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Okoh, O.O., Sadimenko, A. P., Afolayan, A. J. (2010). Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chemistry*, 120 (1), 308-312.

Ould el hadj, M., Hadj mahammed, M., Zabeiro, H. (2003). Place des plantes spontanées dans la médicine traditionnelle de la région de Ouargla (Sahara septentrional est). *Courrier du Savoir*, 47-51.

Ozenda, P. (2004). Flore et végétation du Sahara. (3rd ed.). CNRS, 663.

Pushpangadan, P., George, V. (2012). Handbook of Herbs and Spices. 2ndEds, Vol. 1, Woodhead Publishing Series in Food Science, *Technology and Nutrition*, 55-72.

Quezel, P., Santa, S. (1962). Nouvelle flore de l'Algérie et des régions désertiques. (CNRS ed.). (pp. 1170).

Smaili, T., Zellagui, A. Gherraf, N., Flamini, G., Cioni, P. L. (2011). Essential oil content of the flowers of *Pituranthos Scoparius* in Algeria Medicinal plant, *International Journal of Phytomedicines and Related Industries*, 3(2), 177-179.

Vernin, G., Ghiglione, C., Mostafa, D., Parkanyi, C. (1999). GC/MS Analysis of volatile constituents of essential oils of *Pituranthos Scoparius*, *Journal of essential oil research*, 11, 673-676.

Vérité, P., Nacer, A. (2003). Composition of seeds and stems and essential oils of *Pituranthos Scoparius (Coss. & Dur.)* Schinz. *Flavour and Fragrance Journal*, 19, 562-564.

Wang, Z., Ding, L., Li, T., Zhou, X., Wang, L., Zhang, H.Q. (2006). Improved solvent-free microwave extraction of essential oil from dried *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim. *Journal of Chromatography A*, 1102(1-2), 11-17.

**Acknowledgements**

We would like to especially thank the Department of Pharmacotoxicology–Saidal of Medea, Algeria, for laboratory materials support and for hosting the pharmacological activities in their laboratories.