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## Chemical compositions of essential oils and Hydrosol extract of *Schinus Molle Linens* and assessment of its antioxidant activity

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**ABSTRACT** : *Schinus molle* L., commonly known as the false pepper tree, belongs to the Anacardiaceae family. Essential oils extracted from the leaves and seeds of the plant using hydrodistillation revealed differences in composition compared to the hydrosol extract obtained via liquid-liquid extraction. The yields for the essential oils (leaves, seeds) and hydrosol extract were 1.62%, 2.78%, and 0.196%, respectively. Gas chromatography-mass spectrometry (GC-MS) analysis identified 75 compounds in the leaf oils and 63 in the seed oils in the leaf and seed essential oils, respectively. The major compounds in the leaf essential oils include  $\alpha$ -Phellandrene (20.45%),  $\beta$ -Phellandrene (13.68%), Dehydroxy-Isocalamendiol (11.66%), (-)-Germacrene D (8.93%) and  $\delta$ -Cadinene (5.46%). For the seed essential oils, the main components were  $\alpha$ -Phellandrene (31.21%),  $\beta$ -Phellandrene (23.64%),  $\beta$ -Myrcene (7.8%), Dehydroxy-Isocalamendiol (4.82%) and  $\delta$ -Cadinene (4.60%). The analysis of the hydrosol extract was analyzed separately by GC-MS for the first time and identified 52 compounds, with major components including  $\delta$ -Cadinene (9.45%),  $\alpha$ -Cadinol (7.13%), Elemol (6.28%), cis-Sabinol (5.89%),  $\alpha$ -Eudesmol (5.15%),  $\tau$ -Muurolol (5.10%), (-)-Germacrene D (4.80%), 2,6-di-tert-butyl-4-methyl-phenol (4.56%) and D-Limonene (4.14%). Antioxidant activity evaluation using three chemical methods revealed remarkable free radical scavenging and reducing capacities. The leaf essential oils showed the highest activity in DPPH tests (2.605  $\mu$ g/ml to scavenge 50% of free radicals), although lower than vitamin C and FRAP tests (EC50 of 7.48 10-4 $\mu$ g/ml), with competitive CAT results. The seed essential oils, while less effective than the leaf oils, exhibited high efficiency in the CAT method (1.635 mg/g) and closely matched vitamin C in FRAP tests.

**Keywords:** *Schinus molle*, essential oils, hydrosol extract, GC-MS, antioxidant activities, South West Algeria.

## 1. INTRODUCTION

As part of efforts to valorize Algerian flora, this work focuses on analyzing the chemical composition of essential oils and hydrosol extract derived from *Schinus molle* and evaluating their antioxidant properties. Approximately 300–400 essential oils are used commercially out of the 3,000 known varieties (Van de Braak et al, 1999). Plant hydrosols may possess therapeutic properties distinct from their corresponding essential oils (Price et al., 2004).

*Schinus molle*, a member of the *Anacardiaceae* family (Cherroud, 2021). *Schinus Molle* is used to treat various diseases such as pain, asthma, infections, and other conditions. It has analgesic, antihemorrhagic, antibacterial, antifungal qualities (Malea-Gareia et al., 2017; Temesgen Bekele et al., 2023). Antiseptic, stimulant, and wound-healing properties (Salem et al., 2016). Previous studies have reported that the EOs of *Schinus Molle* are characterized by their a antioxidant, antimicrobial, cytotoxicity, and insecticidal properties (Machado et al., 2019).

This study, part of a broader research effort to preserve and utilize this species, analyzes the composition of essential oils extracted from leaves, seeds, and hydrosol extract of *S. molle* and investigates their antioxidant properties.

## 2. MATERIAL AND METHODS

### 2.1. Extraction of EOs

The plant material (leaves and seeds of the false pepper tree) was collected from the Béchar (South West Algeria) region (latitude 31.5° N, longitude -2.23°, altitude 773 m). The flowering period of *Schinus molle* L. occurs in February. The essential oils were obtained via hydrodistillation using a Clevenger-type apparatus for approximately 3 hours, following the procedure outlined in the European Pharmacopoeia (Council of Europe, 1997). The oils were stored at +4°C before being used for various tests.

### 2.2 Extraction of the Hydrosol Extract

Following the hydrodistillation process for extracting essential oils from the leaves and seeds, 800 ml of hydrosol extract were collected. The volatile compounds were extracted using successive liquid-liquid extractions with diethyl ether. After drying with anhydrous magnesium sulfate, filtering, and evaporating the solvent under reduced pressure, a concentrated oily residue was obtained at a concentration of 1.795mg/ml relative to the initial volume. The hydrosol was stored at +4°C prior to use.

### 2.3 Characterization by Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The analysis of the samples was carried out at the Technical Platform for Physico-Chemical Analyses (PTAPC-CRAPC) in Laghouat, Algeria. The samples were analyzed using a gas chromatograph coupled to a mass spectrometer, SHIMADZU GCMS-QP2020, equipped with a Rxi®-5ms capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane). A volume of 0.5 µL of the sample diluted in n-hexane was injected. The injector and detector temperatures were set at 250°C and 310°C, while the column temperature was programmed from 50°C to 310°C. Helium was used as the carrier gas at a flow rate of 1 mL/min. Different components of the EO were identified by their retention indices (RI) compared to a mixture of linear alkanes nC8-C33 and by comparing their mass spectra with those of two mass spectral libraries (Wiley version 11 and Nist version 17). The RIs were also validated by comparison with literature values (Babushok et al., 2011; Adams, 2017). If you want to calculate the retention indices, you should use the following (Kovats formula):

$$LRI = 100 \times \left( \frac{t_x - t_n}{t_{n+1} - t_n} \right) + 100 \times n \quad (1)$$

Where:

**LRI:** Linear Retention Indices.

**t<sub>x</sub>:** Retention time of the targeted sample component.

**t<sub>n</sub>:** Retention time of the alkane C<sub>n</sub> (lower n-alkane).

**t<sub>n+1</sub>:** Retention time of the alkane C<sub>n+1</sub> (higher n-alkane). The n-alkane series used in this analysis was (n-C8-C33).

## 2.4 Antioxidant Activity Study

### 2.4.1 Total Antioxidant Capacity

This test evaluates the reduction of molybdenum (VI) to molybdenum (V) by plant extracts, forming a green phosphate/Mo (V) complex under acidic conditions. A sample (100 µL) of essential oil diluted in methanol was reacted with 1ml of a solution containing sulfuric acid (0.6 N), sodium phosphate (28 mM) and ammonium molybdate (4 mM). After incubating the tubes at 95°C for 90 minutes and cooling to room temperature, absorbance was measured at 695 nm relative to a methanol blank. Results were expressed in milligrams of ascorbic acid equivalents per gram of extract (Prieto et al., 1999).

### 2.4.2 Iron Reduction: Frap (Ferric Reducing Antioxidant Power)

The FRAP (Ferric Reducing Antioxidant Power) method measures the ability of an antioxidant to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>. This change is visible by the change from yellow (Fe<sup>3+</sup>) to blue-green (Fe<sup>2+</sup>), and the intensity of the color is measured at 700 nm. The protocol, according to Yildirim et al. (2000), consists of mixing 0.5 ml of essential oil at different concentrations with 1.25 mL of a 0.2 M phosphate buffer solution (pH = 6.6) and 1.25mL of potassium ferricyanide K<sub>3</sub>Fe (CN)<sub>6</sub> at 1%. Then, the mixture is incubated at 50 °C for 20 minutes, cooled, then 2.5 ml of 10% trichloroacetic acid are added to stop the reaction. After centrifugation at 3000 tpm for 10 min, 1.25 mL of the supernatant is mixed with distilled water and 0.1% iron chloride (FeCl<sub>3</sub>, 6H<sub>2</sub>O). Absorbances are read at 700 nm. The median effective concentration (EC<sub>50</sub>) is determined for an absorbance of 0.5, obtained by the interpretation of the linear regression curve (OD = f ([C])) and compared to the activity of ascorbic acid.

### 2.4.3 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Free Radical Scavenging

The preliminary evaluation of the antioxidant activity of the samples was carried out using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, which was initially developed by Blois (1958). DPPH, violet in color in solution, has an absorption maximum at 515 nm. The antioxidant power of the tested EOs was estimated by comparison with a natural antioxidant (ascorbic acid). All tests were performed with three repetitions for each concentration. The DPPH radical scavenging activity was measured according to the protocol described by Lopes-Lutz et al (2008), where 100µL of each of the methanolic

solutions of the tested EOs at different concentrations; In order to prepare the stock solutions of each EO, 80  $\mu\text{L}$  of EO are added to 4 mL of methanol, to obtain a final concentration of the stock solution of 20  $\mu\text{L}/\text{mL}$ . From the stock solutions, different concentrations of essential oils were prepared (10  $\mu\text{L}/\text{mL}$ ; 5  $\mu\text{L}/\text{mL}$ ; 2.5  $\mu\text{L}/\text{mL}$ ; 1.25  $\mu\text{L}/\text{mL}$ ; 0.625  $\mu\text{L}/\text{mL}$ ; 0.312  $\mu\text{L}/\text{mL}$  and 0.156  $\mu\text{L}/\text{mL}$ ). The control used is ascorbic acid which is prepared at the same concentration as the essential oils, these are mixed with 1300  $\mu\text{L}$  of a methanoic solution of DPPH (0.004%). After an incubation period of 30 minutes at laboratory temperature, the absorbance is read at 517 nm. The inhibition of the DPPH free radical by ascorbic acid was also analyzed for comparison. The percentage inhibition of free radicals (PI%) was calculated using the following formula:

$$\text{PI \%} = [1 - (\text{Ab}_{\text{Stest}}/\text{Ab}_{\text{Scontrol}})] \times 100 \quad (2)$$

**Ab<sub>test</sub>**: absorbance of the sample.

**Ab<sub>control</sub>**: absorbance of the negative control.

All tests were performed in triplicate.

IC<sub>50</sub> (inhibitory concentration 50%), also known as EC<sub>50</sub> (Efficient concentration 50), is the concentration required to reduce the DPPH radical by 50%. IC<sub>50</sub> values are calculated graphically using percentage inhibition curves from different extract concentrations (Torres et al., 2006).

### 3. RESULTS AND DISCUSSION

#### 3.2 Identification by GC-MS of Essential Oils and Hydrosol Extract

To our knowledge, the South West of Algeria is the first place where the chemical makeup of the essential oils and hydrosol extract of the False pepper tree has been determined (W. Béchar). Table 1 provide a summary of the various components of the extracted essential oils as found in the leaves, seeds and hydrosol's extract.

**Table 1.** Chemical compositions of EOs from *S. molle* Leaves, Seeds and Hydrosol extract.

| Identified Compounds              |                                 | Leaves          |                      | Seeds           |                      | Hydrosol extract |                      |
|-----------------------------------|---------------------------------|-----------------|----------------------|-----------------|----------------------|------------------|----------------------|
|                                   |                                 | <sup>a</sup> RI | <sup>b</sup> Area(%) | <sup>a</sup> RI | <sup>b</sup> Area(%) | <sup>a</sup> RI  | <sup>b</sup> Area(%) |
| <b>Peak N°</b>                    | <b>Hydrocarbon monoterpenes</b> |                 |                      |                 |                      |                  |                      |
| 1                                 | Tricyclene                      | 729             | 0.04                 | -               | -                    | -                | -                    |
| 2                                 | $\alpha$ -Thujene               | 927             | 0.01                 | -               | -                    | -                | -                    |
| 3                                 | 2-Pinene                        | 948             | 1.89                 | 948             | 1.51                 | -                | -                    |
| 4                                 | Camphene                        | 943             | 0.28                 | 943             | 0.06                 | -                | -                    |
| 5                                 | Sabinene                        | 972             | 0.07                 | 972             | 0.04                 | -                | -                    |
| 6                                 | $\beta$ -Pinene                 | 943             | 0.13                 | 943             | 0.08                 | -                | -                    |
| 7                                 | $\beta$ -Murène                 | 958             | 1.99                 | 958             | 7.48                 | 987              | 1.09                 |
| 8                                 | $\alpha$ -Phellandrene          | 969             | 20.45                | 969             | 31.21                | 1008             | 2.31                 |
| 9                                 | $\alpha$ -Terpinene             | 1018            | 0.01                 | 1018            | 0.02                 | -                | -                    |
| 10                                | p-Cymene                        | 1025            | 0.99                 | 1025            | 2.28                 | -                | -                    |
| 11                                | o-Cymene                        | -               | -                    | -               | -                    | 1026             | 1.53                 |
| 12                                | D-Limonene                      | -               | -                    | -               | -                    | 1032             | 4.14                 |
| 13                                | $\beta$ -Phellandrene           | 964             | 13.68                | 964             | 23.64                | -                | -                    |
| 14                                | $\gamma$ -Terpinene             | 998             | 0.02                 | 998             | 0.02                 | -                | -                    |
| 15                                | Terpinolene                     | 1086            | 0.07                 | 1086            | 0.14                 | -                | -                    |
| <b>Total</b>                      |                                 | <b>39,63</b>    |                      | <b>66,48</b>    |                      | <b>9.07</b>      |                      |
| <b>Monoterpene alcohols</b>       |                                 |                 |                      |                 |                      |                  |                      |
| 16                                | Linalool                        | -               | -                    | 1101            | 0.02                 | 1098             | 0.71                 |
| 17                                | Isomyrcenol                     | -               | -                    | 1072            | 0.04                 | -                | -                    |
| 18                                | 2-p-Menthen-1-ol                | -               | -                    | -               | -                    | 1130             | 0.37                 |
| 19                                | trans-, para-Menth-2-en-1-ol    | 1124            | 0.02                 | 1124            | 0.08                 | -                | -                    |
| 20                                | $\alpha$ -Phellandrene-8-ol     | -               | -                    | -               | -                    | 1177             | 0.36                 |
| 21                                | P-Cymen-7-ol                    | -               | -                    | -               | -                    | 1191             | 0.90                 |
| 22                                | Cis-Carveol (Carveol II)        | -               | -                    | 1109            | 0.04                 | -                | -                    |
| 23                                | L- $\alpha$ -Terpineol          | -               | -                    | -               | -                    | 1202             | 0.27                 |
| 24                                | Terpinen-4-ol                   | 1137            | 0.02                 | 1137            | 0.05                 | -                | -                    |
| 25                                | $\gamma$ -Isogeraniol           | -               | -                    | -               | -                    | 1224             | 0.60                 |
| 26                                | P- Menthan-2-ol, 1,8-epoxy-     | -               | -                    | -               | -                    | 1233             | 1.31                 |
| 27                                | Cis-Sabinol                     | 1085            | 0.02                 | 1085            | 0.31                 | 1213             | 7.29                 |
| 28                                | Camphenol, 6-                   | 1131            | 0.02                 | -               | -                    | -                | -                    |
| 29                                | Piperitone                      | -               | -                    | -               | -                    | 1272             | 0.42                 |
| 30                                | Phellandrel                     | -               | -                    | -               | -                    | 1290             | 0.60                 |
| 31                                | Thymol                          | -               | -                    | -               | -                    | 1300             | 1.67                 |
| 32                                | Carvacrol                       | 1317            | 0.03                 | 1317            | 0.09                 | -                | -                    |
| 33                                | Pinanediol                      | 1276            | 0.10                 | 1276            | 0.08                 | -                | -                    |
| <b>Total</b>                      |                                 | <b>0,21</b>     |                      | <b>0,71</b>     |                      | <b>14.5</b>      |                      |
| <b>Oxygenated monoterpenes</b>    |                                 |                 |                      |                 |                      |                  |                      |
| 34                                | Bornyl acetate                  | 1277            | 0.04                 | 1277            | 0.04                 | -                | -                    |
| 35                                | Isoascaridol                    | 1011            | 0.1                  | 1011            | 0.08                 | -                | -                    |
| 36                                | Ascaridol                       | -               | -                    | -               | -                    | 1272             | 0.41                 |
| 37                                | Cryptone                        | -               | -                    | -               | -                    | 1196             | 0.97                 |
| 38                                | Geranyl ester                   | 1550            | 0.01                 | 1550            | 0.20                 | -                | -                    |
| 39                                | Sobrerol 8-acetate              | -               | -                    | 1521            | 0.03                 | -                | -                    |
| <b>Total</b>                      |                                 | <b>0,15</b>     |                      | <b>0,35</b>     |                      | <b>1.38</b>      |                      |
| <b>Hydrocarbons Sesquiterpene</b> |                                 |                 |                      |                 |                      |                  |                      |
| 40                                | $\delta$ -Elemene               | 1335            | 0.04                 | -               | -                    | -                | -                    |
| 41                                | $\alpha$ -Cubebene              | 1344            | 0.03                 | 1344            | 0.04                 | -                | -                    |
| 42                                | Copaene                         | 1221            | 0.23                 | 1221            | 0.12                 | 1391             | 0.39                 |
| 43                                | Caryophyllene "V1"              | 1407            | 0.04                 | -               | -                    | -                | -                    |

|     |   |      |              |      |              |      |              |
|-----|---|------|--------------|------|--------------|------|--------------|
| 44  | Elemene                                 | 1398 | 0.72         | 1398 | 0.73         | -    | -            |
| 45  | $\alpha$ -Gurjunene                     | 1419 | 0.89         | 1419 | 0.69         | 1425 | 1.00         |
| 46  | Caryophyllene                           | 1494 | 1.01         | 1494 | 1.26         | 1439 | 1.02         |
| 47  | (-)- $\beta$ -copaene                   | 1339 | 0.02         | -    | -            | -    | -            |
| 48  | Germacrene B                            | 1603 | 0.02         | -    | -            | 1580 | 0.28         |
| 49  | Aromadendrene                           | 1438 | 0.03         | -    | -            | -    | -            |
| 50  | Cadina-3,5-diene                        | 1472 | 0.18         | 1472 | 0.05         | -    | -            |
| 51  | $\alpha$ -Caryophyllene                 | 1579 | 0.71         | 1579 | 0.52         | 1475 | 0.88         |
| 52  | Epi- $\beta$ -Caryophyllene             | 1494 | 0.29         | 1494 | 0.22         | -    | -            |
| 53  | Longifolen-v2                           | 1280 | 0.36         | -    | -            | -    | -            |
| 54  | $\gamma$ -Muuroleone                    | -    | -            | 1489 | 0.28         | 1489 | 1.54         |
| 55  | (-)-Germacrene D                        | 1515 | 8.93         | 1515 | 1.95         | 1500 | 4.80         |
| 56  | $\beta$ -Selinene                       | 1469 | 0.76         | 1469 | 0.66         | -    | -            |
| 57  | (-)-Dehydroaromadendrene                | 1362 | 0.12         | 1362 | 0.09         | -    | -            |
| 58  | Bicyclosesquiphellandrene               | -    | -            | 1435 | 0.18         | -    | -            |
| 59  | $\beta$ -Chamigrene                     | -    | -            | 1507 | 0.38         | -    | -            |
| 60  | $\beta$ -Cubebéne                       | 1339 | 0.39         | -    | -            | -    | -            |
| 61  | $\gamma$ -Gurjunene                     | -    | -            | 1461 | 0.28         | -    | -            |
| 62  | Bicyclogermacrene                       | 1499 | 1.71         | -    | -            | -    | -            |
| 63  | $\alpha$ -Muuroleone                    | 1440 | 0.75         | 1440 | 0.76         | -    | -            |
| 64  | $\alpha$ -Elemene                       | -    | -            | -    | -            | 1536 | 0.51         |
| 65  | $\beta$ -Elemene                        | 1398 | 0.37         | 1398 | 0.15         | 1398 | 0.87         |
| 66  | $\gamma$ -Cadinene                      | -    | -            | 1512 | 0.91         | -    | -            |
| 67  | $\delta$ -Cadinene                      | 1518 | 5.46         | 1518 | 4.60         | 1532 | 9.45         |
| 68  | Cubenene                                | 1440 | 0.09         | 1440 | 0.08         | 1547 | 0.26         |
| 69  | $\alpha$ -Amorphene                     | 1440 | 0.12         | -    | -            | 1551 | 0.46         |
| 70  | Germacrene B                            | -    | -            | -    | -            | 1580 | 0.28         |
| 71  | $\alpha$ -Calacorene                    | 1547 | 0.04         | -    | -            | -    | -            |
| 72  | trans-Calamenene                        | 1537 | 0.05         | -    | -            | -    | -            |
| 73  | Silphiperfol-4,7(14)-diene              | 1409 | 0.09         | -    | -            | -    | -            |
| 74  | Daucene                                 | -    | -            | -    | -            | 1728 | 0.30         |
| 75  | $\gamma$ -Maaliene                      | 1398 | 0.16         | -    | -            | -    | -            |
| 76  | $\alpha$ -Phellandrene, dimer           | 1746 | 0.04         | -    | -            | -    | -            |
|     | <b>Total</b>                            |      | <b>23,65</b> |      | <b>13,97</b> |      | <b>21,76</b> |
|     | <b>Sesquiterpene alcohols</b>           |      |              |      |              |      |              |
| 77  | 2,6-Di-tert-butyl-4-methyl-phenol (BHT) | -    | -            | -    | -            | 1509 | 4.56         |
| 78  | $\alpha$ -Cadinol                       | 1580 | 3.10         | -    | -            | 1673 | 7.13         |
| 79  | Cubebol                                 | 1484 | 1.48         | -    | -            | -    | -            |
| 80  | Shyobunol                               | 1555 | 2.25         | 1555 | -            | 1718 | 2.52         |
| 81  | Elemol                                  | -    | -            | -    | -            | 1561 | 6.28         |
| 82  | $\alpha$ -Elemol                        | 1546 | 3.33         | 1546 | 1.74         | -    | -            |
| 83  | Cedr-8(15)-en-9-ol                      | 1586 | 0.14         | -    | -            | -    | -            |
| 84  | Palustrol                               | 1530 | 0.35         | 1530 | 0.04         | -    | -            |
| 85  | Germacren- D-4-ol                       | 1660 | 1.30         | 1660 | 1.08         | 1594 | 3.01         |
| 86  | Ledol                                   | -    | -            | -    | -            | 1627 | 1.43         |
| 87  | Gleenol                                 | 1630 | 0.05         | 1630 | 0.04         | -    | -            |
| 88  | Viridiflorol                            | 1594 | 0.69         | 1594 | 0.34         | 1616 | 1.32         |
| 89  | Dehydroxy-isocalamendiol                | 1586 | 11.66        | 1586 | 4.82         | -    | -            |
| 90  | Isospathulenol                          | 1569 | 0.07         | 1569 | 0.06         | -    | -            |
| 91  | Kusenol                                 | 1626 | 0.07         | -    | -            | -    | -            |
| 92  | Cubenol                                 | -    | -            | -    | -            | 1632 | 0.52         |
| 93  | Epicubenol                              | -    | -            | -    | -            | 1645 | 0.65         |
| 94  | Epiglobulol                             | -    | -            | -    | -            | 1695 | 0.34         |
| 95  | $\alpha$ -Eudesmol                      | -    | -            | -    | -            | 1672 | 5.15         |
| 96  | $\gamma$ -Eudesmol                      | 1632 | 2.34         | -    | -            | -    | -            |
| 97  | $\tau$ -Cadinol                         | 1580 | 2.73         | 1580 | 1.44         | -    | -            |
| 98  | $\alpha$ , epi-Muurolol                 | 1580 | 0.41         | -    | -            | -    | -            |
| 99  | $\alpha$ -Muurolol                      | -    | -            | 1580 | 0.18         | -    | -            |
| 100 | $\tau$ -Muurolol ( $\delta$ -Cadinol)   | -    | -            | 1580 | 2.60         | 1660 | 5.10         |
| 101 | 15-Cpaenol                              | -    | -            | 1464 | 0.13         | -    | -            |
| 102 | $\beta$ -Eudesmol                       | 1656 | 1.60         | 1656 | 0.75         | 1650 | 2.30         |
| 103 | (-)-Spathulenol                         | -    | -            | -    | -            | 1685 | 1.21         |

|     |   |      |              |      |              |      |              |
|-----|---|------|--------------|------|--------------|------|--------------|
| 104 | 1-Naphthalenol,1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-,(1S,4S) | 1687 | 0.03         | -    | -            | -    | -            |
| 105 | Bulnesol  | 1614 | 0.19         | -    | -            | -    | -            |
| 106 | Maaliol   | -    | -            | -    | -            | 1703 | 0.64         |
| 107 | $\beta$ -Copaen-4-ol  | -    | -            | -    | -            | 1710 | 0.73         |
| 108 | Oplopanon   | -    | -            | -    | -            | 1753 | 0.73         |
| 109 | Isocalamenediol   | 1725 | 1.73         | 1725 | 0.38         | 1771 | 3.19         |
| 110 | Proximadiol   | -    | -            | -    | -            | 1840 | 1.94         |
| 111 | Platambin   | -    | -            | -    | -            | 1909 | 0.29         |
|     | <b>Total</b>  |      | <b>33,52</b> |      | <b>15,44</b> |      | <b>49.04</b> |
|     | <b>Oxygenated sesquiterpenes</b>  |      |              |      |              |      |              |
| 112 | Isoshoybunone   | 1571 | 0.18         | 1571 | 0,16         | -    | -            |
| 113 | Caryophyllene oxide   | -    | -            | 1507 | 0.23         | 1604 | 0.48         |
| 114 | 9H-Cycloisolongifolene, 8-oxo-  | 1368 | 0.02         | -    | -            | -    | -            |
|     | <b>Total</b>  |      | <b>0,20</b>  |      | <b>0,39</b>  |      | <b>0.48</b>  |
|     | <b>Oxygenated Diterpenes</b>  |      |              |      |              |      |              |
| 115 | Fortunolide A   | 2386 | 0.03         | -    | -            | -    | -            |
|     | <b>Total</b>  |      | <b>0,03</b>  |      | -            |      | -            |
|     | <b>Hydrocarbon Diterpenes</b>   |      |              |      |              |      |              |
| 116 | $\alpha$ -Phellandrene, dimer   | -    | -            | 1746 | 0.07         | -    | -            |
| 117 | m-Camphorene  | -    | -            | 1982 | 0.12         | -    | -            |
| 118 | p-Camphorene  | -    | -            | 1982 | 0.03         | -    | -            |
|     | <b>Total</b>  |      | -            |      | <b>0,22</b>  |      | -            |
|     | <b>Oxygenated Triterpenes</b>   |      |              |      |              |      |              |
| 119 | Mollugogenol E  | 2955 | 2.32         | -    | -            | -    | -            |
|     | <b>Total</b>  |      | <b>2,32</b>  |      | -            |      | -            |
|     | <b>Hydrocarbon monoterpenes</b>   |      | 39,63        |      | 66,48        |      | 9.07         |
|     | <b>Monoterpene alcohols</b>   |      | 0,21         |      | 0,71         |      | 14.5         |
|     | <b>Oxygenated monoterpenes</b>  |      | 0,15         |      | 0,35         |      | 1.38         |
|     | <b>Total Monoterpenes</b>   |      | <b>39,99</b> |      | <b>67,54</b> |      | <b>24.95</b> |
|     | <b>Hydrocarbon sesquiterpenes</b>   |      | 23,65        |      | 13,97        |      | 21.76        |
|     | <b>Sesquiterpene alcohols</b>   |      | 33,52        |      | 15,44        |      | 49.04        |
|     | <b>Oxygenated sesquiterpenes</b>  |      | 0,2          |      | 0,39         |      | 0.48         |
|     | <b>Total Sesquiterpenes</b>   |      | <b>57,37</b> |      | <b>29,80</b> |      | <b>71.28</b> |
|     | <b>Oxygenated diterpenes</b>  |      | 0,03         |      | -            |      | -            |
|     | <b>Hydrocarbon diterpenes</b>   |      | -            |      | 0,22         |      | -            |
|     | <b>Total Diterpenes</b>   |      | <b>0,03</b>  |      | <b>0,22</b>  |      | -            |
|     | <b>Oxygenated triterpenes</b>   |      | 2,32         |      | -            |      | -            |
|     | <b>Total Terpenes</b>   |      | <b>99,71</b> |      | <b>97,56</b> |      | <b>96.23</b> |

<sup>a</sup> Retention index

<sup>b</sup> the relative percentages of the constituents (%) are calculated on the basis of the areas of the GC peaks measured on the appolar column (Rxi@-5ms)

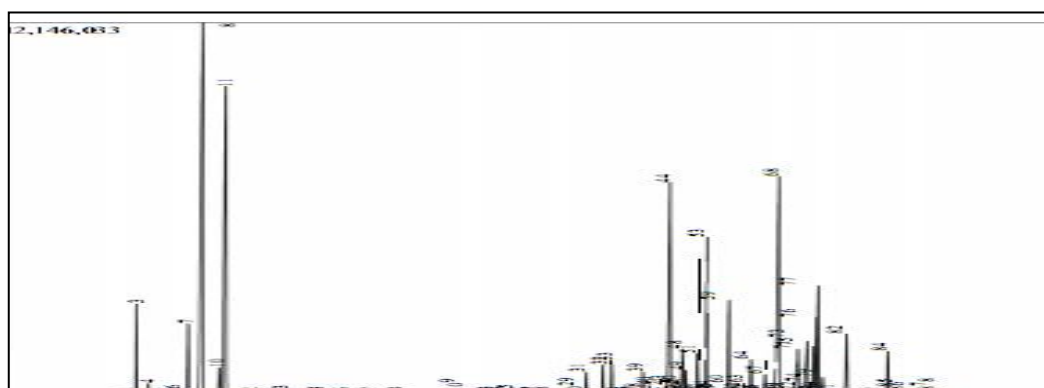
### 3.2.1 Discussion

The chromatographic analysis carried out on the mixture of essential oils (leaves, seeds and Hydrosol extract) of the false pepper tree allowed us to identify 119 compounds. Most of the constituents of the EOs are terpenes. Their proportion exceeds 97%. The main major constituents obtained EOs (leaves, seeds) in our study were  $\alpha$ -Phellandrene with (20.45%; 31.21%), and  $\beta$ -Phellandrene with (13.68%; 23.64%) (Table1).

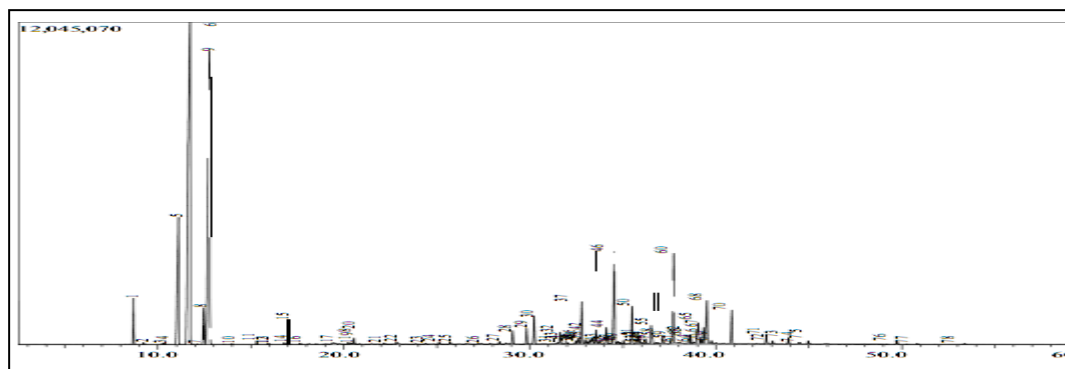
► The EOs of the leaves have almost the same chromatographic profile as that of the seeds. The main compounds of the leaf EOs were:  $\alpha$ -Phellandrene (20.45%),  $\beta$ -Phellandrene (13.68%), Dehydroxy-isocalamendiol (11.66%), (-)- Germacrene D (8.93%) and  $\delta$ -Cadinene (5.46%),  $\alpha$ -Elemol (3.33%),  $\alpha$ -Cadinol (3.10%),  $\tau$ -Cadinol (2.73%),  $\gamma$ -Eudesmol (2.34%), Mollugogenol (2.32%) (Figure 1).

► The main compounds of the seed EOs were:  $\alpha$ -phellandrene (31.21%),  $\beta$ -Phellandrene (23.64%),  $\beta$ -Myrcene (7.8%), Dehydroxy-Isocalamendiol (4.82%)  $\delta$ -Cadinene (4.60%),  $\tau$ -Muurolol ( $\delta$ -Cadinol) (2.60%), para-Cymene (2.28%), (-)-Germacrene D (1.95%) and  $\alpha$ -Elemol (1.74%) (Figure 2).

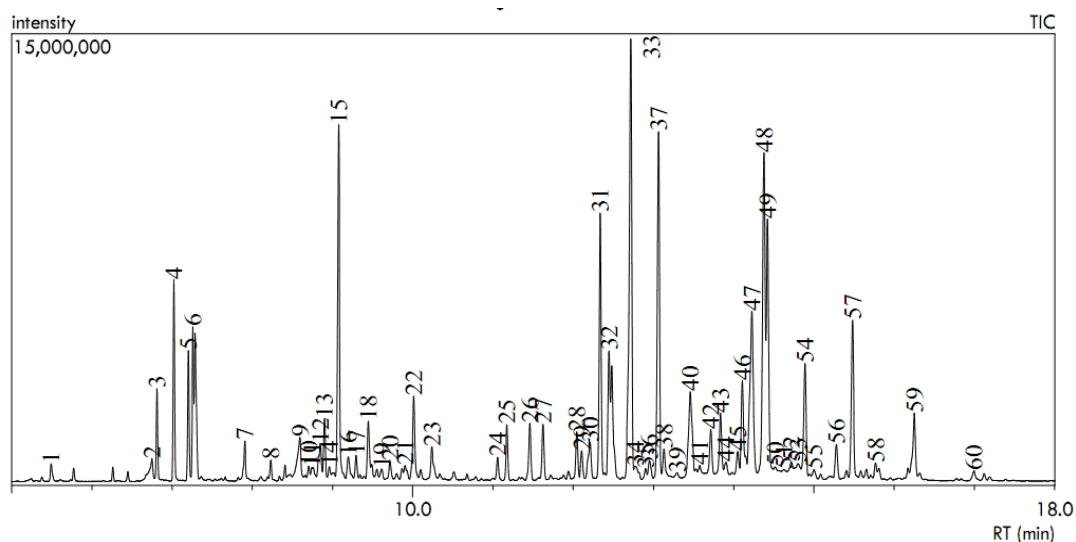
► The composition of the hydrosol extract was low concentrations of the essential oil and they contain traces of aromatic compounds and water-soluble molecules that differ from those present in the essential oil, offering a unique and complementary profile. The major compounds were:  $\delta$ -Cadinene (9.45%),  $\alpha$ -Cadinol (7.13%), Elemol (6.28%), cis-Sabinol (5.89%),  $\alpha$ -Eudesmol (5.15%),  $\tau$ -Muurolol (5.10%), (-)-Germacrene D (4.80%), 2,6-Di-tert-butyl-4-methyl-phenol (4.56%), D-Limonene (4.14%) (Figure 3).



**Figure. 1.** Chromatogram of EOs from *S. Molle* Leaves (SML).



**Figure. 2.** Chromatogram of EOs from *S. Molle* Seeds (SMS).



**Figure 3.** Chromatogram of EOs from *S. Molle* Hydrosol (SMH).

### 3.3 Comparison Of The Chemical Composition Of The Essential Oils Of The Leaves, Seeds and That Of The Hydrosol Extract Of False Pepper Tree

A thorough GC-MS comparison would show the parallels and particular differences between these chemical compositions of the false pepper tree's essential oils;

Based on comparing their mass spectra and retention indices with those of genuine ones from various libraries, 75 constituents in all, accounting for over 99% of the composition of the terpenic essential oil of *Schinus molle* leaves, were identified (Table 1). This oil is distinguished by its substantial concentration of sesquiterpene fraction, which accounts for roughly 57.37% of its total chemical composition and of which sesquiterpene alcohols make up 33.52%, hydrocarbon sesquiterpenes make up 23.65% and oxygenated sesquiterpenes make up 0.20 percent. Additionally, this oil was distinguished by its concentration of monoterpene fraction, which accounts for approximately 39.99% of its total chemical composition and of which hydrocarbon monoterpenes are the primary constituents. Of the substances found,  $\beta$ -Phellandrene was the most significant at 39.63% (13.68%), followed by monoterpene alcohols at 0.21%, oxygenated monoterpenes at 0.15%, and oxygenated triterpenes at 2.32%, which were found in little amounts. Additionally, only small amounts of oxygenated diterpenes (0.03%) make up the diterpenes fraction. According to Table 1, a total of 63 constituents, representing more than 97% of the composition of the terpenic essential oil of *Schinus molle* seeds. These essential in contrast to 0.71% for monoterpene alcohols and 0.35% for oxygenated monoterpenes, oils were distinguished by their substantial content in the total monoterpene fraction, which accounts for roughly 67.54% of the oil's overall chemical composition. Of these, hydrocarbon monoterpenes make up 66.48% of the

oil's main constituents, with  $\beta$ -Phellandrene accounting for the most significant amount at 23.64 percent. Additionally, this oil was distinguished by its significant concentration of the total sesquiterpene fraction, which makes up around 29.80% of its total chemical composition. Of this, hydrocarbon sesquiterpenes make up 13.97% and sesquiterpene alcohols 15.44%. Additionally, only hydrocarbon diterpenes (0.22%) make up the diterpene fraction.

Table 1 show that the terpenic essential oil of the hydrosol extract of *Schinus molle* contains 52 constituents in total, accounting for over 96% of its composition. Sesquiterpene alcohols are the primary ingredients of these essential oils, which are distinguished by their substantial content in the total sesquiterpene fraction, which accounts for roughly 71.28% of the oil's overall chemical composition. 49.04%; hydrocarbon sesquiterpenes make up 21.76%, of which  $\delta$ -Cadinene was the most significant (9.45%); and oxygenated sesquiterpenes make up 0.48%. appeared in small proportion. against 9.07% for hydrocarbon monoterpenes, 14.5% for monoterpene alcohols and 1.38% for oxygenated monoterpenes.

When compared to hydrocarbon monoterpenes, which make up 66.48% of the main constituents, leaves' essential oils (EOs) make up 39.63%, hydrosol makes up 49.04 percent of the main constituents of sesquiterpene alcohols and seeds' oils have a high and significant content of hydrocarbon monoterpenes.

The high percentage of oxygenated compounds in leaves EOs was 2.7% by weight, compared to 1.86% for hydrosol extract and 0.74% for seed EOs. Furthermore, there can be variations in the amount and presence of certain terpenes, alcohols, hydrocarbons and other particular chemical substances among them, suggesting different chemical profiles. Similar significant chemicals, such as  $\alpha$ -Phellandrene and  $\beta$ -Phellandrene, were present in both leaves.

The hydrosol extract also exhibits a wide range of compounds, with  $\delta$ -Cadinene and  $\alpha$ -Cadinol comprising 9.45% and 7.13%, respectively. These findings underscore the chemical diversity of the essential oils and hydrosol extract derived from the leaves and seeds of *S. molle*.

While the chemical profile of the essential oils aligns closely with reports in scientific literature, certain qualitative and quantitative variations were observed. For instance, a study focusing on samples from different regions of Algeria, specifically the Blida region,

indicates that the essential oils of the false pepper tree contain 67 identified compounds, predominantly terpenes exceeding 60% of the total composition. The main components in the leaf essential oils were  $\alpha$ -Phellandrene (24.32%), Limonene (21.62%),  $\beta$ -Myrcene (17.76%), and  $\alpha$ -Pinene (4.13%). On the other hand, the seed essential oils primarily consisted of  $\alpha$ -Phellandrene (23.28%), Limonene (21.04%), 3-Carene (17.35%) and  $\alpha$ -Pinene (3.98%) (Rouibi et al., 2010). Notable differences include the presence of Limonene in place of  $\beta$ -Phellandrene.

El-Akram et al. (2008) reported that essential oils of *S. molle* from Tunisia were predominantly composed of monoterpenes, making up 95.67% of their total content. The major compounds in these oils include  $\alpha$ -Phellandrene (35%), Phellandrene (29.30%),  $\beta$ -Pinene (15.68%), p-Cymene (5.43%) and  $\alpha$ -Pinene (5.22%).

Similarly, a study by Oumayma et al. (2022) on essential oils extracted from leaves and seeds of *S. molle* collected in Rabat, Morocco, highlights a hydrocarbon monoterpene fraction ranging between 49.70% and 42.65%. The primary constituents were Limonene (18.49%-22.94%),  $\alpha$ -Terpinene (8.15%-12.01%),  $\gamma$ -Terpinene (8.15%-12.01%), Longifolene (8.48%-7.67%), and 10-epi-Elemol (8.03%-7.64%). Interestingly,  $\alpha$ -Phellandrene was present in only trace amounts (0.75%-0.22%). Overall, research on various species of the false pepper tree highlights notable variability in the chemical composition of their essential oils. These differences can be attributed to factors such as plant variety, growth stage, ecological conditions, and extraction techniques.

### 3.4 Results of the Antioxidant Activity Studies

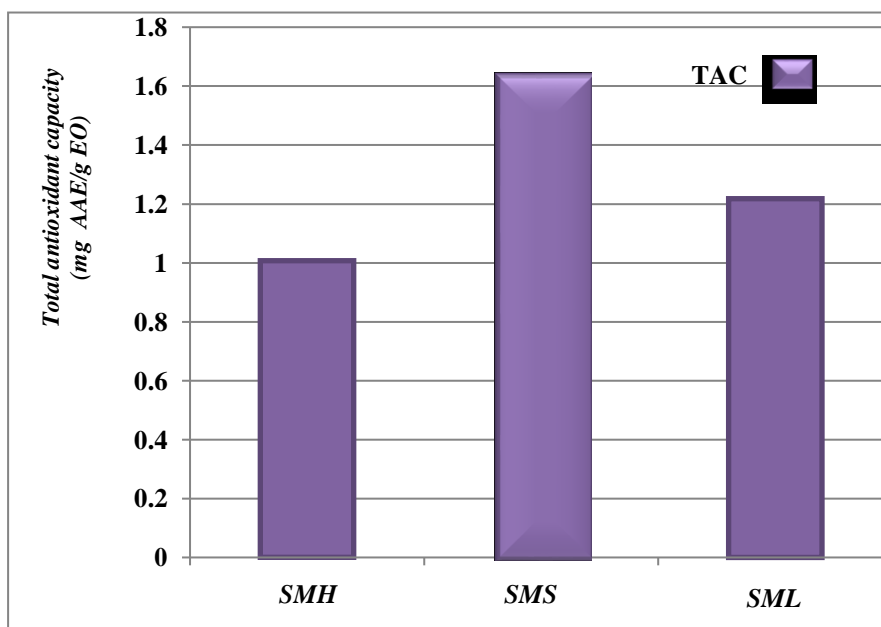
The antioxidant activity of essential oils was evaluated by three methods, namely, the total antioxidant capacity (TAC), the DPPH free radical scavenging, and the iron reduction test (FRAP), from the graphical representations it was possible to measure the critical values represented in the following table 4:

**Table 4.** The antioxidant activities of the samples.

| Sample           | Total antioxidant capacity (mg EAA/g EO) (TAC) | IC <sub>50</sub> / DPPH (µg/mL) 50% Inhibition Concentration | EC <sub>50</sub> / Iron reduction (µg/mL) |
|------------------|--|--|---|
| <i>SML</i>       | 1,218  | 2,605  | 7,48 10 <sup>-4</sup>                     |
| <i>SMS</i>       | 1,635  | 6,30   | 17,1 10 <sup>-3</sup>                     |
| <i>SMH</i>       | 1,007  | 129,53   | 0,35                                      |
| Acide ascorbique | /  | 0,81   | 5,63 10 <sup>-3</sup>                     |

### 3.3.1 Total Antioxidant Capacity

The total antioxidant capacity of the three essential oils exceeds 1mg ascorbic acid equivalent/g. The seed essential oil is the most active at 1.635 mg/g, followed by the leaf essential oil, while the hydrosol extract shows the lowest antioxidant activity (Figure 5).

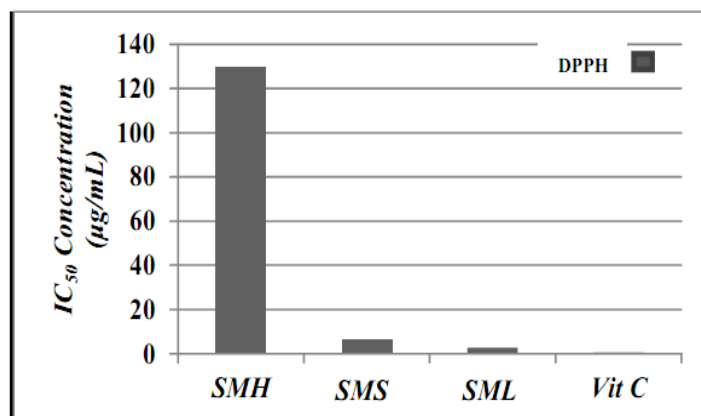


**Figure 5.** Concentrations of essential oils and Hydrosol (mg EAA/g EO) according to the TAC method

TAC: Total antioxidant capacity (mg AAE/g EO: mg ascorbic acid equivalent/g essential oil)

### 3.3.2 DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging

Antioxidant activity, measured by DPPH radical scavenging capacity, increased with concentration for all samples tested, showing a dose-dependent relationship typical of antioxidants. Ascorbic acid had the highest scavenging activity at 20  $\mu\text{g/mL}$  (83.1%) and the lowest  $\text{IC}_{50}$  of 0.81  $\mu\text{g/ml}$ , making it the most potent antioxidant. leaf EOs exhibits better antioxidant activity than seed EOs and Hydrosol extract, with an inhibition rate index of 75% at 20  $\mu\text{g/mL}$  and an  $\text{IC}_{50}$  of 2.605  $\mu\text{g/mL}$ . seed EOs displays moderate activity with a 57% and an  $\text{IC}_{50}$  of 6.30  $\mu\text{g/ml}$ , indicating intermediate efficacy, lower than leaf EOs but higher than Hydrosol extract. Hydrosol extract exhibits the lowest activity, with an  $\text{IC}_{50}$  of 129.53  $\mu\text{g/mL}$ .

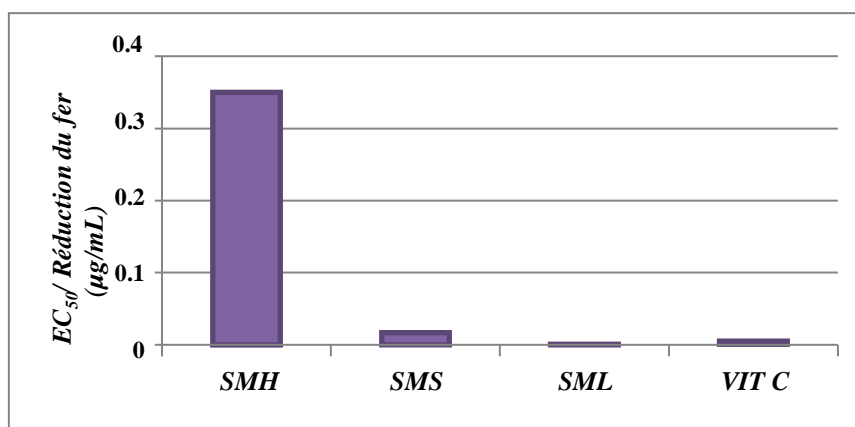


**Figure 6.** IC<sub>50</sub> of essential oils and hydrosol according to DPPH method.

The DPPH free radical scavenging capacity, from the results of the Table we can see that the essential oil of the leaves is the most active with a concentration of 2.605 µg/mL to scavenge 50% of the DPPH free radical, although less compared to vitamin C, followed by the essential oil of the seeds with an IC<sub>50</sub> of 6.30 µg/mL, while the least active hydrosol extract with an IC<sub>50</sub> exceeds 129 µg/mL (Figure 6).

### 3.3.3 Iron Reduction: FRAP (Ferric Reducing Antioxidant Power)

Moving on to iron reduction, the essential oil of the leaf essential oil still remains the most capable of reducing this ion with an EC<sub>50</sub> of  $7.48 \cdot 10^{-4}$  µg/mL stands out with the lowest EC<sub>50</sub> value, indicating a higher antioxidant efficacy (6.69 times more effective than vitamin C). The hydrosol extract has a much higher EC<sub>50</sub>, making it less effective. Vitamin C and the seed essential oil are relatively close, with a slight superiority for vitamin C (1.42 times), but both the leaf essential oil and the seed essential oil are more active than the hydrosol extract. The leaf essential oil being the more active of the two. The histogram shown in (Figure 7) which better shows the most active essential.



**Figure 7.** The EC<sub>50</sub> of essential oils and the hydrosol based on the FRAP method.

### 3.3.3.1 Discussion

The seed essential oil demonstrates strong free radical neutralization capabilities, primarily due to its high concentrations of bioactive compounds such as  $\alpha$ -Phellandrene and  $\beta$ -Phellandrene. In contrast, the leaf essential oil exhibits comparatively lower efficacy, and the hydrosol extract antioxidant potential could be improved by increasing the concentration of active molecules.

The antioxidant power of essential oils varies significantly depending on their chemical composition. With capacities exceeding 1 mg of ascorbic acid equivalent per gram, further research is needed to evaluate their effectiveness and stability under real-world conditions.

Different antioxidant capacities are observed in *Schinus molle* essential oil samples depending on the method used. The leaf essential oil performs exceptionally well in DPPH and FRAP assays and remains competitive in TAC tests, making it a promising candidate for use as a natural preservative or dietary supplement. While the seed essential oil is less effective than the leaf oil in some tests, it excels in TAC and FRAP evaluations. The hydrosol extract, however, shows lower antioxidant activity across the board.

The antioxidant effects of essential oils can be attributed to key compounds such as  $\delta$ -Cadinene, D-Limonene, and 2,6-Di-tert-butyl-4-methyl-phenol (BHT) in the hydrosol extract, as well as Caryophyllene and (-)-Germacrene D in the leaf and seed oils. The high levels of monoterpene hydrocarbons in the essential oils, including active compounds like  $\gamma$ -Terpinene and 2-Pinene, also contribute to their antioxidant properties (Menichini et al., 2009). Oxygenated sesquiterpenes play a role in antioxidant activity, acting individually or synergistically, facilitated by the free electrons from their high oxygenation levels (Abd-Elgawad et al., 2020). Research further suggests that sesquiterpenes possess anti-radical properties, with their structure and concentration influencing their antioxidant mechanisms (Bartikova et al., 2014).

#### 4. CONCLUSIONS

The study enabled the characterization of the chemical composition of *Schinus molle* (*Anacardiaceae*) essential oils (EOs) extracted from leaves, seeds and hydrosol extract using GC-MS analysis. These oils were found to be predominantly terpenic, with terpenes making up more than 97% of their composition, highlighting their potential as novel sources of natural antioxidants agents. Seed EOs rich in monoterpenic hydrocarbons, which account for 66.48% of the composition. Major constituents include  $\alpha$ -Phellandrene (31.21%),  $\beta$ -Phellandrene (23.64%),  $\beta$ -Myrcene (7.8%), Dehydroxy-Isocalamendiol (4.82%) and  $\delta$ -Cadinene (4.60%). Leaf EOs primarily composed of hydrocarbon monoterpenes (39.63%), featuring  $\alpha$ -Phellandrene (20.45%),  $\beta$ -Phellandrene (13.68%), Dehydroxy-Isocalamendiol (11.66%), (-)-Germacrene D (8.93%) and  $\delta$ -Cadinene (5.46%). And Hydrosol extract dominated by sesquiterpene alcohols (49.04%), including  $\delta$ -Cadinene (9.45%),  $\alpha$ -Cadinol (7.13%), Elemol (6.28%), cis-Sabinol (5.89%),  $\alpha$ -Eudesmol (5.15%), tau-Muurolol (5.10%), (-)-Germacrene D (4.80%), 2,6-di-tert-butyl-4-methyl-phenol (4.56%) and D-Limonene (4.14%).

Antioxidant Activity the essential oils from the leaves exhibited strong antiradical and reducing capacities, performing well in DPPH and FRAP tests but still less effective than vitamin C. The seed oils showed significant efficacy in the CAT assay. These variations are attributed to the chemical composition of the oils, suggesting an antioxidant potential exceeding 1mg of ascorbic acid per gram, warranting further study.

This research enhances understanding of the chemical constituents of *Schinus molle* essential oils and supports the valorization of plants used in traditional medicine, contributing to the preservation of plant biodiversity. Future research will focus on evaluating antioxidant activity through various methods, in vivo studies of these potentials, assessing the use of essential oils as natural antimicrobials, exploring other bioactivities of *S. molle* extracts, and analyzing their variability and biological effects in greater detail.

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