applied sciences

MDPI

*Article*

# Chemical Analysis of Essential Oils of *Cymbopogon schoenanthus* (L.) Spreng. and *Nepeta azurea* R.Br. ex Benth from Djbouti, In-Vitro Cytotoxicity against Cancer Cell Lines and Antibacterial Activities

**Fatouma Mohamed Abdoul-Latif [1,*], Abdirahman Elmi [1,2], Ali Merito [1], Moustapha Nour [1], Arnaud Risler [2], Ayoub Ainane [3], Jérôme Bignon [4] and Tarik Ainane [3]**

[1] Medicinal Research Institute, Centre d'Etudes et de Recherche de Djibouti, IRM-CERD, Route de l'Aéroport, Haramous B.P. 486, Djibouti City, Djibouti
[2] Laboratoire Lorrain de Chimie Moléculaire (L2CM), Université de Lorraine, CNRS, F-54000 Nancy, France
[3] Superior School of Technology of Khenifra, University of Sultan Moulay Slimane, BP 170, Khenifra 54000, Morocco
[4] Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, Avenue de la Terrasse, CEDEX, F-91198 Gif-sur-Yvette, France
* Correspondence: fatoumaabdoulatif@gmail.com

**Abstract:** The aim of this research was to evaluate the essential oils of two medicinal plants from Djibouti: *Cymbopogon schoenanthus* (L.) Spreng. and *Nepeta azurea* R.Br. ex Benth. in cytotoxic activity against thirteen (13) cancer cell lines (A2780, A549, HCT116, HEK-293, JIMT-T1, K562, MIA-Paca2, MRC5, NCI-N87, PC3, RT4, U2OS and U87-MG) and antibacterial activity against seven (7) Gram (−) strains (*Acinetobacter baumannii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* sv. *Typhimurium* and *Shigella sonnei*) and five (5) Gram (+) strains (*Corynebacterium* sp., *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae*). The plants were extracted by hydrodistillation and were analyzed by GC-MS. The main components of *Cymbopogon schoenanthus* essential oil (CSEO) were 3-isopropenyl-5-methyl-1-cyclohexene (32.3%) and D-Limonene (11.3%), and the main component of *Nepeta azurea* essential oil (NAEO) was methyl (2E)-2-nonenoate (53.2%). The two essential oils showed inhibitory cytotoxicity activities of all the cancer cells tested; on the other hand, the antibacterial activities are only well-noticed for the CSEO oil at a concentration of 5% against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Corynebacterium* sp., *Enterococcus faecalis* and *Staphylococcus aureus*. Our results demonstrated that the two essential oils could be effective natural anticancer agents, in addition to an antibacterial character for *Cymbopogon schoenanthus* essential oil.

**Keywords:** essential oils; *C. schoenanthus*; *N. azurea*; compositions; biological activities



**Citation:** Mohamed Abdoul-Latif, F.; Elmi, A.; Merito, A.; Nour, M.; Risler, A.; Ainane, A.; Bignon, J.; Ainane, T. Chemical Analysis of Essential Oils of *Cymbopogon schoenanthus* (L.) Spreng. and *Nepeta azurea* R.Br. ex Benth from Djbouti, In-Vitro Cytotoxicity against Cancer Cell Lines and Antibacterial Activities. *Appl. Sci.* **2022**, *12*, 8699. https://doi.org/10.3390/app12178699

Academic Editor: Ramona Iseppi

Received: 11 August 2022
Accepted: 27 August 2022
Published: 30 August 2022



## 1. Introduction

Noncommunicable diseases have a considerable impact on human health, as well as on the economy [1]. They decrease the quality of life of patients and increase the causes of premature death and lead to other harmful consequences [2]. Among these diseases is cancer, which is the second leading cause of death in the world after heart disease. Cancer is a large group of chronic diseases and can target tissues or organs in the human body [3,4]. Currently, cancer treatments are based on several methods, generally based on chemotherapy, radiotherapy, surgery and the use of active ingredients [5,6]. The latter have high costs given the stage and grade of the cancer. However, the use of traditional herbal treatments has gained attention in recent years [7].

Medicinal plants have played a transversal role in the treatment of cancer. Several authors have reported over 3000 species that possess anticancer activities [8–12]. The study

of anticancer agents from natural sources began in the early fifties, when medicinal plants were exploited as a focal point of bioactive molecules and, at the same time, the production of new molecules similar to natural chemical compounds for cancer with different reactions in organic synthesis and sometimes by computer modeling [13,14]. The different parts of plants contain a set of low molecular weight substances called secondary metabolites such as terpenoids, phenolic compounds, flavonoids, alkaloids and other organic compounds substituted by other elements such as sulfur and phosphorus [15–18].

Djibouti is a developing country facing socioeconomic problems with infectious diseases, particularly certain types of cancerous diseases, in addition to the lack of medical technology for screening and diagnosis, which are mostly unaffordable for the population, and the execution preventive and awareness programs in marginal areas [19]. Djibouti is known for its wide variety of climatic conditions, including a tropical desert on the coast and in the north, while it becomes a semi-desert at the central heights. The Djiboutian flora is one of the few species studied in the world, and a large number of medicinal plants have been used by the local population to fight against diseases, particularly cancer [20–22].

The current study was conducted in the regions of Mouloud and Day in Djibouti, from where the populations of these suburbs claim that cancer has been treated using dozens of medicinal plants since antiquity. The present study therefore has to evaluate two medicinal plants: *Cymbopogon schoenanthus* and *Nepeta azurea* used in the treatment of cancer and in the treatment of other pathogenic diseases, because they have the potential to be evaluated and used in the development of pharmaceutical products useful for the future.

## 2. Material and Methods

### *2.1. Collection and Identification of Vegetable Material*

The collection of plant materials took place in two different areas of Djibouti: the first plant of *Cymbopogon schoenanthus* was collected from the Mouloud region (southwest of Djibouti; 11°10′18.3″ N 42°30′01.4″ E), and the second plant of *Nepeta azurea* was collected from the Day region (north of Djibouti; 11°44′46.6″ N 42°41′03.5″ E) (Figure 1). The collection period included February and March. The two species were identified by the first author, and they are classified in an herbarium of the Medicinal Research Institute of Djibouti (CERD) with the respective access numbers CS4-2019 and NA3-2019.


**Figure 1.** Harvest locations of two species: *Cymbopogon schoenanthus* and *Nepeta azurea* in the Republic of Djibouti.

### *2.2. Extraction of Essential Oils*

Both CSEO and NAEO essential oils from *Cymbopogon schoenanthus* and *Nepeta azurea* were extracted by the Clevenger apparatus. The dried aerial parts were cut into small pieces, and 50 g of each species were immersed in 400 mL of distilled water and heated

under reflux for 4 h. After dehydration of the organic phase with anhydrous sodium sulphate, the volumes of the essential oils were determined, and the viscous liquids were stored in amber glass bottles wrapped in aluminum foil at 4 °C [23]. All experiments were performed in triplicate.

The yields of NOEC and NAEO were determined by the following formula:

$$Y(\%) = \left(\frac{V}{W} \times 100\right) \pm \left(\frac{\Delta V}{W} \times 100\right)$$

with

$V$: volume of essential oil collected;
$\Delta V$: volume uncertainty;
$W$: weight of dry vegetable matter.

### 2.3. GC-MS Analyses

The identification of the chemical composition of two essential oils CSEO and NAEO was made using an apparatus of gas chromatography coupled to a mass spectrometer equipped with a flame ionization detector (FID) (Agilent Technologies 7820, Santa Clara, CA, USA). All the components and the properties of use are mentioned in Figure 2 [24].

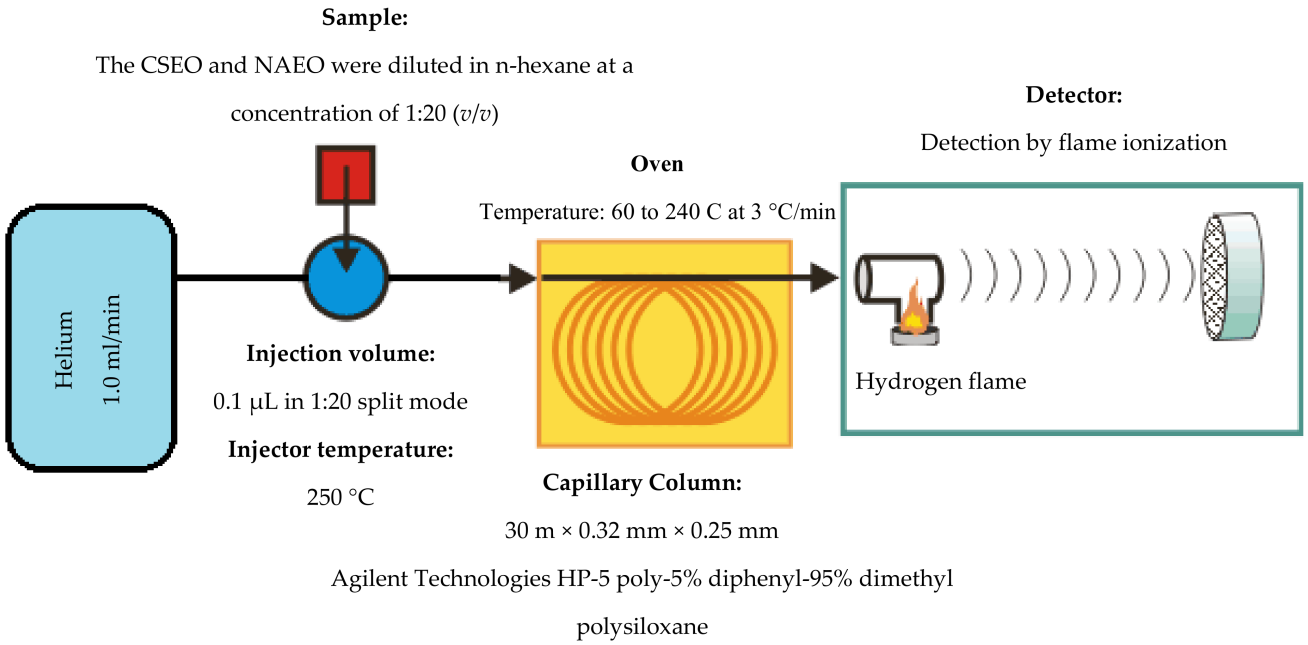


**Figure 2.** Equipment components and operating conditions of the GC-MS analysis.

### 2.4. Cytotoxicity Assay

All cancer cell lines (Table 1) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), the German Collection of Microorganisms and Cell Cultures of the Leibniz Institute (DSMZ, Braunschweig, Germany) and the European Collection of Authenticated Cell Cultures (ECACC, England). Each cell type was cultured in its appropriate medium and supplemented with 10% fetal bovine serum, 1% glutamine and 1% penicillin/streptomycin. Additionally, the cell lines were maintained at 5% $CO_2$. The cultures were periodically examined under an inverted microscope to assess the absence of microbial contaminants and the amount of confluence.

**Table 1.** The origin, source and growth medium of 13 cancer cell lines used in the cytotoxicity test.

| Cell Lines | Origin | Source | Growth Medium |
|---|---|---|---|
| A2780 | *Ovarian carcinoma* | ECACC-93112517 | Gibco RPMI 1640 |
| A549 | *Lung carcinoma* | ATCC®-CCL-185TM | Gibco RPMI 1640 |
| HCT116 | *Colorectalcarcinoma* | ATCC®-CCL-247TM | Gibco McCoy's 5A |
| HEK-293 | *Embryonic kidney* | ATCC®-CRL-1573TM | Gibco RPMI 1640 |
| JIMT-T1 | *Breast carcinoma* | DSMZ-ACC 589 | Gibco DMEM |
| K562 | *Myelogenous leukemia* | ATCC®-CCL-243TM | Gibco RPMI 1640 |
| MIA-Paca2 | *Pancreas carcinoma* | ATCC®-CRL-1420TM | Gibco DMEM |
| MRC5 | *Lung normal* | ATCC®-CCL-171TM | Gibco DMEM |
| NCI-N87 | *Gastric carcinoma* | ATCC®-CRL-5822TM | Gibco RPMI 1640 |
| PC3 | *Prostate carcinoma* | ATCC®-CRL-1435TM | Gibco RPMI 1640 |
| RT4 | *Urinary bladder* | ATCC®-HTB-2TM | Gibco McCoy's 5A |
| U2OS | *Bone osteosarcoma* | ATCC®-HTB-96TM | Gibco McCoy's 5A |
| U87-MG | *Brain glioblastoma* | ATCC®-HTB-14TM | Gibco DMEM |

Both CSEO and NAEO essential oils were tested in ELISA microplates at different concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01 and 0.005 µg/mL; solvent DMSO 5%), where 10 µL of each concentration was added to 90 µL of culture medium, which contained $3.10^3$ cells/wells. After the incubation of microplates for 72 h, 100 µL of CellTiter Glo Reagent was added to each well for 15 min before recording the luminescence with a spectrophotometric plate reader. The dose–response curves were plotted with Excel software, and the 50% inhibition concentration values ($IC_{50}$) were determined from the polynomial trend curves [25].

### 2.5. Antibacterial Activity

The antibacterial activity was studied according to the effect of the essential oils against Gram-negative and Gram-positive bacteria (Table 2) by determining the diameters of inhibition using the agar well diffusion test [26]. This method is widely used for the evaluation of the antibacterial activity of essential oils. In this manipulation, the surfaces of the petri dishes were inoculated by spreading 100 µL of the bacterial suspension over the entire surface of the Mueller-Hinton Medium agar. Then, four perforations were made (diameter: 6 mm; depth: 5 mm), one for the negative control (dimethylsulfoxide DMSO 5%), one for the positive control (commercial antibiotics + DMSO 5%), one for CSEO and the last for NAEO. It is noted that the two essential oils were tested at a concentration of 5% in DMSO, and three repetitions were made per test and for each strain. The antibacterial activity was evaluated according to the zone of inhibition visualized after incubation of the petri dishes for 24 h at 37 °C.

**Table 2.** The strains used during the antibacterial test.

| Gram Negative | | Gram Positive | |
|---|---|---|---|
| **Strains** | **Code/Source** | **Strains** | **Code/Source** |
| *Acinetobacter baumannii* | ATCC 19606 | | |
| *Enterobacter cloacae* | Clinical isolate | *Corynebacterium* sp. | Clinical isolate |
| *Escherichia coli* | ATCC 25922 | *Enterococcus faecalis* | ATCC 29212 |
| *Klebsiella pneumoniae* | ATCC 700603 | *Staphylococcus aureus* | ATCC 29213 |
| *Pseudomonas aeruginosa* | ATCC 27853 | *Staphylococcus epidermidis* | Clinical isolate |
| *Salmonella enterica* | ATCC 13311 | *Streptococcus agalactiae* | ATCC 27956 |
| *Shigella sonnei* | *ATCC 9290* | | |

## 3. Statistical Analysis

The data from all tests were statistically evaluated using XLSTAT software. The values were presented as the mean ± uncertainty at 95% of the three replicates of each experiment using Student's Law.

## 4. Results

After the harvesting of the medicinal plants *C. schoenanthus* and *N. azurea*, the subject of this research in the two regions of Djibouti, extractions by hydrodistillation using a Clevenger apparatus, was carried out to obtain, respectively, the two essential oils CSEO and NAEO. The yields of these two essential oils obtained were, respectively, 1.16% and 1.25% (Table 3).

**Table 3.** Yields of both essential oils.

| Essential Oil | Yield (%) |
|---|---|
| CSEO | 1.16 ± 0.22 |
| NAEO | 1.25 ± 0.25 |

The chemical compositions of the CSEO and NAEO were determined by gas chromatography (GC) coupled with a flame ionization detector (FID); all the data found are mentioned in Table 4. The analyses made allowed us to identify 47 compounds for CSEO with a total percentage of 99.9%; among them, two (2) major compounds were visualized: 3-isopropenyl-5-methyl-1-cyclohexene (32.3%) and D-limonene (11.3%) and seven (7) compounds with moderate concentrations between 2% and 8%, such as: β-elemol (7.8%), 2-p-menthen-1-ol (5.1%), geraniol acetate (3.7%), terpineol, cis-beta.- (3.5%), 10-epi-β-eudesmol (3.0%), 4-Nonanone (2.1%) and cis-piperitol (2.1%). In addition, the NAEO analysis identified 20 compounds with a percentage of 100%, hence a single major compound, methyl (2E)-2-nonenoate (53.2%), and seven (7) compounds with concentrations moderate between 2% and 9%, such as: phellandral (8.6%), 1-methyl-2-decalone (6.1%), cis-caryophyllene (5.7%), cyclohexanol acetate (5.0%), boschnialactone (3.6%), caryophyllene oxide (2.8%) and L-calamenene (2.0%). The two oils have compounds in common with different concentrations; we noted: 1-tetradecyne, elemene, cis-caryophyllene, methyl (2E)-2-nonenoate, caryophyllene oxide and viridiflorol.

**Table 4.** Chemical compositions of CSEO and NAEO.

| Pic | RT (*) | Compounds | CSEO | NAEO |
|---|---|---|---|---|
| 1 | 6.35 | 3-Isopropenyl-5-methyl-1-cyclohexene | **32.3** | - |
| 2 | 6.51 | α-Phellandrene | 0.2 | - |
| 3 | 7 | β-Phellandrene | 0.5 | - |
| 4 | 7.1 | D-Limonene | **11.3** | - |
| 5 | 7.3 | β-trans-Ocimene | 1.7 | - |
| 6 | 7.54 | Ocimene | 1.1 | - |
| 7 | 8.15 | 4-Nonanone | 2.1 | - |
| 8 | 8.49 | L-Fenchone | 0.5 | - |
| 9 | 8.75 | Linalol | - | 0.4 |
| 10 | 9.24 | 2-p-Menthen-1-ol | 5.1 | - |
| 11 | 9.49 | cis-p-menth-2-en-1-ol | 0.2 | - |
| 12 | 9.6 | Terpineol, cis-.beta.- | 3.5 | - |
| 13 | 10.21 | Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl- | 0.8 | - |

**Table 4.** *Cont*.

| Pic | RT (*) | Compounds | CSEO | NAEO |
|---|---|---|---|---|
| 14 | 10.39 | Anethofuran | 0.7 | - |
| 15 | 10.58 | α-Terpineol | 8.5 | - |
| 16 | 10.7 | α-Phellandren-8-ol | 0.7 | - |
| 17 | 10.823 | cis-Piperitol | 2.1 | - |
| 18 | 10.9 | trans-Piperitol | 1.4 | - |
| 19 | 11.09 | gamma.-Isogeraniol | 0.6 | - |
| 20 | 11.22 | cis-Geraniol | 0.4 | - |
| 21 | 11.55 | piperitone | - | 1.7 |
| 22 | 11.85 | 4-Undecanone | 0.3 | - |
| 23 | 11.95 | 2-Caren-10-al | 0.3 | - |
| 24 | 12.13 | 2-Undecanone | - | 0.8 |
| 25 | 12.14 | Cyclohexene, 2-ethenyl-1,3,3-trimethyl- | 0.2 | - |
| 26 | 12.27 | 1-Tetradecyne | 0.3 | 0.8 |
| 27 | 12.34 | 2-Acetoxy-1,8-cineole | 0.4 | - |
| 28 | 12.56 | Boschnialactone | - | 3.6 |
| 29 | 12.87 | Isogeraniol | - | 1.7 |
| 30 | 12.95 | Neryl acetate | 0.5 | - |
| 31 | 13.07 | Geraniol acetate | 3.7 | - |
| 32 | 13.31 | Limonene oxide | - | 1 |
| 33 | 13.43 | β-Bourbonene | - | 0.6 |
| 34 | 13.5 | Elemene | 0.8 | 0.5 |
| 35 | 13.68 | 1-Methyl-2-decalone | - | 6.1 |
| 36 | 13.81 | Oplopanone | - | 1.8 |
| 37 | 13.93 | Cis-caryophyllene | 0.8 | 5.7 |
| 38 | 13.99 | Cyclohexanol, acetate | - | 5 |
| 39 | 14.09 | α-Bergamotene | 0.2 | - |
| 40 | 14.57 | Inknown | - | 0.9 |
| 41 | 14.63 | 4-Dodecanone, 11-methyl- | 0.3 | - |
| 42 | 14.74 | Phellandral | - | 8.6 |
| 43 | 14.74 | Germacrene D | 0.3 | - |
| 44 | 14.86 | β-eudesmene | 0.2 | - |
| 45 | 14.94 | Methyl (2E)-2-nonenoate | 0.2 | **53.2** |
| 46 | 15.15 | τ-cadinene | 0.3 | - |
| 47 | 15.2 | δ-cadinene | 0.6 | - |
| 48 | 15.25 | L-calamenene | - | 2 |
| 49 | 15.59 | β- elemol | 7.8 | - |
| 50 | 16.03 | Caryophyllene oxide | 1.2 | 2.8 |
| 51 | 16.31 | Cis-carveol | 0.2 | - |
| 52 | 16.41 | Cubenol | - | 1.5 |
| 53 | 16.52 | Eudesm-7(11)-en-4-ol | 1.4 | - |

**Table 4.** *Cont.*

| Pic | RT (*) | Compounds | CSEO | NAEO |
|---|---|---|---|---|
| 54 | 16.61 | γ-eudesmol | 0.7 | - |
| 55 | 16.72 | τ-cadinol | 0.6 | - |
| 56 | 16.89 | 10-epi-β-eudesmol | 3 | - |
| 57 | 16.97 | Viridiflorol | 0.7 | 1.3 |
| 58 | 17.18 | Trans-2-Hydroxy-1,8-cineole | 0.2 | - |
| 59 | 17.49 | Eudesma-4,11-dien-2-ol | 0.3 | - |
| 60 | 17.68 | longipinocarveol | 0.3 | - |
| 61 | 18.69 | Eudesm-5-en-11-ol | 0.4 | - |
| | | Total (%) | **99.9** | **99.1** |

(*) RT: Retention time.

The results of the in vitro cytotoxicity test against the thirteen (13) cancer cell lines (A2780, A549, HCT116, HEK-293, JIMT-T1, K562, MIA-Paca2, MRC5, NCI-N87, PC3, RT4, U2OS and U87-MG) are expressed by the 50% inhibition concentrations ($IC_{50}$) in Table 5. After reading the values obtained and comparing them with positive standards such as: Vinblastine, Doxorubicie, Combrestatin A4 and Monomethyl Auristatin E, the two essential oils have interesting activities against all the cell lines tested, and in many cases, were more effective than the standards tested.

**Table 5.** $IC_{50}$ (μg/mL) values for the evaluation of CSEO and NAEO in the cytotoxicity test against the cancer lines.

| Cell Line | CSEO | NAEO | Vinblastine | Doxorubicie | Combrestatin A4 | MMAE (*) |
|---|---|---|---|---|---|---|
| A2780 | 0.14 ± 0.03 | 0.62 ± 0.09 | - | - | - | 0.45 ± 0.01 |
| A549 | 0.49 ± 0.23 | 0.07 ± 0.01 | - | 56.60 ± 0.84 | 20.00 ± 0.10 | 0.46 ± 0.05 |
| HCT116 | 0.65 ± 0.03 | 0.11 ± 0.01 | 35.00 ± 0.84 | - | 2.00 ± 0.10 | 2.07 ± 0.02 |
| HEK293 | 0.19 ± 0.05 | 0.83 ± 0.11 | - | - | - | - |
| JIMT-T1 | 1.50 ± 0.30 | 2.07 ± 0.20 | - | - | - | - |
| K562 | 0.99 ± 0.01 | 1.00 ± 0.01 | 20.00 ± 0.12 | - | 5.00 ± 0.30 | 3.12 ± 0.20 |
| MIA-Paca2 | 0.55 ± 0.02 | 0.86 ± 0.01 | - | - | - | 4.36 ± 0.20 |
| MRC-5 | 0.83 ± 0.09 | 0.09 ± 0.01 | - | 39.88 ± 1.22 | - | - |
| NCI-N87 | 3.26 ± 1.52 | 0.90 ± 0.27 | - | - | - | 1.65 ± 0.07 |
| PC3 | 0.29 ± 0.01 | 0.80 ± 0.07 | - | 2.09 ± 0.03 | | 0.36 ± 0.03 |
| RT4 | 4.75 ± 1.24 | 0.53 ± 0.01 | - | 36.29 ± 1.20 | - | 0.50 ± 0.01 |
| U2OS | 0.24 ± 0.02 | 0.67± 0.05 | - | - | - | - |
| U87-MG | 0.59 ± 0.09 | 1.13 ± 0.22 | 2.00 ± 0.04 | 99.61 ± 2.34 | 9.00 ± 0.50 | 0.21 ± 0.03 |

(*) MMAE: Monomethyl Auristatin E.

The additional antibacterial activity in this work against twelve (12) bacterial strains of two oils: CSEO and NAEO are mentioned in Table 6. The data set obtained did not give good results. All bacteria were resistant to NAEO at a concentration of 5%; however, CSEO showed activity against only four (4) bacteria: *Corynebacterium* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

**Table 6.** Antibacterial activity of the CSEO and NAEO.

| Gram | Bacterial Strain | CSEO (*) | NAEO (*) |
|---|---|---|---|
| Gram + | *Corynebacterium* sp. | + | - |
| | *Enterococcus faecalis* | - | - |
| | *Staphylococcus aureus* | + | - |
| | *Staphylococcus epidermidis* | - | - |
| | *Streptococcus agalactiae* | - | - |
| Gram − | *Acinetobacter baumannii* | - | - |
| | *Enterobacter cloacae* | - | - |
| | *Escherichia coli* | - | - |
| | *Klebsiella pneumoniae* | + | - |
| | *Pseudomonas aeruginosa* | + | - |
| | *Salmonella enterica* sv. *Typhimurium* | - | - |
| | *Shigella sonnei* | - | - |

(*) Both essential oils tested at 5%. (+) Activity. (-) No activity

## 5. Discussion

To reduce the risk of chronic diseases with regards to the research of treatments based on medicinal plants, essential oils have been explored in recent decades as a natural and safe alternative [27]. In addition to being more expensive, the available treatments also have side effects. The in vitro biological activities of several essential oils in diverse applications have been well-documented by various researchers, particularly anticancer and antimicrobial tests [28–30].

A number of plant species, including *C. schoenanthus* and *N. azurea*, are traditionally used for the treatment of various diseases in Djibouti. In this context, the objective of this study was to evaluate the two essential oils of these two plants using two different in vitro tests: the first concerns the cytotoxicity test of the cancerous cell lines, and the second test targets the antibacterial activities against clinical strains.

The chemical compounds identified in the two essential oils CSEO and NAEO are totally different compared to other previous studies made (Table 7) [31–36]. Several molecules are determined as major compounds in CSEO, particularly piperitone, which presented percentages of 61.01% and 59.80% in the CSEO of African countries such as Togo and Burkina Faso, respectively [32,35]; on the other hand, our study presented 3-isopropenyl-5-methyl-1-cyclohexene (32.3%) and D-limonene (11.3%) as major compounds. Moreover, limonene has been listed as one of the major compounds of CSEO from India, with a percentage of 19.54% [31]. Subsequently, only one search was found on NAEO in Kenya, from where the composition of this oil gave two major compounds, such as: nepetalactone (14.67%) and β-cubebene (10.52%) [36], which are different from our study, which gave methyl (2E)-2-nonenoate (53.2%). These differences in the chemotypes observed in all these studies are due to several factors, among them the meteorological parameters and soil type [37].

In vitro tests against the cancer cell lines of two essential oils: CSEO and NAEO gave promising results, which confirms the traditional use of two medicinal plants *Cymbopogon schoenanthus* and *Nepeta azurea* in Djiboutian herbal medicine. The antitumor properties of essential oils from different species of *Cymbopogon* spp. and *Nepeta* spp. have been the subject of a large number of publications, which have been confirmed by in vitro studies, including *Cymbopogon nardus*, *Cymbopogon flexuosus*, *Cymbopogon citratus*, *Nepeta schiraziana*, *Nepeta sibirica*, *Nepeta rtanjensis*, *Nepeta curviflora* and *Nepeta curvidens* (Table 8) [38–47]. The cytotoxic actions of essential oils against tumor cell lines depend on different types of cancer. Several molecules are endowed with antitumor properties, particularly phenols, alcohols and aldehydes [48]. It is generally the essential oils rich in such molecules that exhibit the greatest cytotoxic efficacy against cancer cell lines. Several mechanisms have been

proposed to explain the reactions involved. Generally, two hypotheses are confirmed for the actions of these compounds by several authors, such as: the power to activate apoptosis in tumor cells and the power to disrupt the mitochondrial membrane potential [49,50].

**Table 7.** Investigation on the chemical compositions of CSEO and NAEO.

| Essential Oils | Investigator | Place | Major Constituents |
|---|---|---|---|
| CSEO | Shahi and Tava (1993) [31] | Jaisalmer (India) | Limonene (19.54%) and 2-undecanone (14.68%) |
| | Ketoh et al. (2005) [32] | Kozah (Togo) | Piperitone (61.01%) and Carene-2 (23.4%) |
| | Katiki et al. (2012) [33] | Sao Paulo (Brazil) | Geraniol (59.42%) and Geranial (13.49%) |
| | Watheq Malti et al. (2020) [34] | Bechar–Ghardaia (Algeria) | Cis-p-menth-2-en-1-ol (22.6-28.5%), trans-p-menth-2-en-1-ol (15.4–16.3%) |
| | Sawadogo et al. (2022) [35] | Ouagadougou (Burkina Faso) | Piperitone (59.8%) and 2-carene (16.4%) |
| NAEO | Simiyu (2004) [36] | Central (Kenya) | nepetalactone (14.67%) and β-cubebene (10.52%) |

**Table 8.** Investigation of the anticancer activity of some essential oils of *Cymbopogon* and *Nepeta* species.

| Essential Oil of Plant | Cancer Cell Lines Tested | Reference |
|---|---|---|
| *Cymbopogon nardus* | Oral (KB) and blood (P388) | Manosroi et al. (2006) [38] |
| *Cymbopogon flexuosus* | Colon (HT-29, HCT-15, SW-620, 502713), lung (A549, HOP-62, H-226), cervix (SiHa), oral (KB), prostate (DU-145) and promyelocytic leukemia (HL-60) | Sharma et al. (2009) [39] |
| *Cymbopogon citratus* | Head and neck (HTB43) | Yen et al. (2016) [40] |
| *Cymbopogon nardus* | Breast (MCF-7), Cervic (HeLa) and myelogenous leukemia (K562). | Sujatha et al. (2019) [41] |
| *Cymbopogon citratus* | Kidneys (VERO) and Cervic (SiHa) | Pan et al. (2022) [42] |
| *Nepeta schiraziana* | Liver tissue (Hep-G2) and breast (MCF-7). | Sharifi-rad et al. (2017) [43] |
| *Nepeta sibirica* | Stomach (HL60, Kato III) | Tsuruoka et al. (2012) [44] |
| *Nepeta rtanjensis* | Cervic (HeLa), myelogenous leukemia (K562), Lung (A549), colon (LS-174) and breast (MDA-MB-231). | Skorić et al. (2017) [45] |
| *Nepeta curviflora* | Cervic (HeLa) | Jaradat et al. (2020) [46] |
| *Nepeta curvidens* | Lung (A549), oral (KB) and prostate (C450). | Ashrafi et al. (2020) [47] |

Finally, the in vitro antibacterial tests of the two essential oils did not show any activity, except the CSEO, which has an activity against the four bacterial strains *Corynebacterium* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These results have been proven by several authors who confirmed the absence of antimicrobial activities of species of the genera *Cymbopogon* spp. and *Nepeta* spp. [51,52].

## 6. Conclusions

Essential oils obtained from *Cymbopogon schoenanthus* and *Nepeta azurea* can be used in the medical field as a natural alternative to replace or reduce the use of synthetic drugs, as they exhibit significant anticancer properties alone and/or in pharmaceutical formulations. These essential oils have shown promising results against dozens of cancer cell lines, as well as *Cymbopogon schoenanthus* essential oil showing antibacterial activity against some clinical strains. The results of the reported studies can be considered as the basis for further in vivo studies.

**Author Contributions:** Conceptualization, F.M.A.-L. and T.A.; methodology, A.E.; software, A.M., M.N., A.R., A.A. and J.B.; validation, F.M.A.-L. and T.A.; formal analysis, A.E. and J.B.; investigation, F.M.A.-L. and A.M.; resources, F.M.A.-L.; data curation, F.M.A.-L.; writing—original draft preparation, A.A.; writing—review and editing, F.M.A.-L.; visualization, T.A.; supervision, A.A.; project administration, F.M.A.-L. and funding acquisition, F.M.A.-L. All authors have read and agreed to the published version of the manuscript.

**Funding:** The authors received no external funding for this article.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of ESTK (accession number: 2019-1215-0003).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data is contained within the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Abdoul-Latif, F.M.; Ainane, A.; Aboubaker, I.H.; Ahmed, N.M.; Ainane, T. Effectiveness of a diet for type 2 diabetics based on vegetables and fruits of the Cucurbitaceae family. *J. Anal. Sci. Appl. Biotechnol.* **2021**, *3*, 107–113.
2. Ainane, T. Moroccan traditional treatment for fever and influenza, similar to symptoms of coronavirus COVID-19 disease: Mini Review. *J. Anal. Sci. Appl. Biotechnol.* **2020**, *2*, 1–3.
3. Elmi, A.; Spina, R.; Risler, A.; Philippot, S.; Mérito, A.; Duval, R.E.; Abdoul-Latif, F.M.; Laurain-Mattar, D. Evaluation of Antioxidant and Antibacterial Activities, Cytotoxicity of Acacia seyal Del Bark Extracts and Isolated Compounds. *Molecules* **2020**, *25*, 2392. [CrossRef]
4. Singh, A.P.; Biswas, A.; Shukla, A.W.; Maiti, P. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. *Signal Transduct. Target. Ther.* **2019**, *4*, 1–21. [CrossRef]
5. Li, Z.; Feiyue, Z.; Gaofeng, L. Traditional Chinese medicine and lung cancer-From theory to practice. *Biomed. Pharmacother.* **2021**, *137*, 111381.
6. Najafi, M.; Majidpoor, J.; Toolee, H.; Mortezaee, K. The current knowledge concerning solid cancer and therapy. *J. Biochem. Mol. Toxicol.* **2021**, *35*, e22900. [CrossRef]
7. Roy, A.; Datta, S.; Bhatia, K.S.; Bhumika; Jha, P.; Prasad, R. Role of plant derived bioactive compounds against cancer. *S. Afr. J. Bot.* **2021**. [CrossRef]
8. Hamedi, A.; Bayat, M.; Asemani, Y.; Amirghofran, Z. A review of potential anti-cancer properties of some selected medicinal plants grown in Iran. *J. Herb. Med.* **2022**, *33*, 100557. [CrossRef]
9. Khajuria, A.K.; Manhas, R.; Kumar, H.; Bisht, N. Ethnobotanical study of traditionally used medicinal plants of Pauri district of Uttarakhand, India. *J. Ethnopharmacol.* **2021**, *276*, 114204. [CrossRef]
10. Abdoul-Latif, F.M.; Elmi, A.; Merito, A.; Nour, M.; Risler, A.; Ainane, A.; Bignon, J.; Ainane, T. Essential Oils of Tagetes minuta and Lavandula coronopifolia from Djibouti: Chemical Composition, Antibacterial Activity and Cytotoxic Activity against Various Human Cancer Cell Lines. *Int. J. Plant Biol.* **2022**, *13*, 315–329.
11. Ali, N.A.A.; Chhetri, B.K.; Dosoky, N.S.; Shari, K.; Al-Fahad, A.J.A.; Wessjohann, L.; Setzer, W.N. Antimicrobial, Antioxidant, and Cytotoxic Activities of Ocimum forskolei and Teucrium yemense (Lamiaceae) Essential Oils. *Medicines* **2017**, *4*, 17. [CrossRef]
12. Khakzad, S.; Rahmani, F.; Hojjati, M.; Tabandeh, M.R. Anti-carcinogenic effects of Satureja khuzistanica and Zataria multiflora essential oils on K562 cell line proliferation. *J. Food Bioprocess Eng.* **2019**, *2*, 127–132.
13. Ainane, T.; Abourriche, A.; Kabbaj, M.; Elkouali, M.; Bennamara, A.; Charrouf, M.; Lemrani, M. Biological activities of extracts from seaweed Cystoseira tamariscifolia: Antibacterial activity, antileishmanial activity and cytotoxicity. *J. Chem. Pharm. Res.* **2014**, *6*, 607–611.
14. Ju, F.; Lee, H.K.; Osarogiagbon, R.U.; Yu, X.; Faris, N.; Li, J. Computer modeling of lung cancer diagnosis-to-treatment process. *Transl. Lung Cancer Res.* **2015**, *4*, 404–414. [CrossRef] [PubMed]

15. Ainane, T.; Elkouali, M.; Ainane, A.; Talbi, M. Moroccan traditional fragrance based essential oils: Preparation, composition and chemical identification. *Der Pharma Chem.* **2014**, *6*, 84–89.
16. Talbi, M.; Saadali, B.; Boriky, D.; Bennani, L.; Elkouali, M.H.; Ainane, T. Two natural compounds—A benzofuran and a phenylpropane–from Artemisia dracunculus. *J. Asian Nat. Prod. Res.* **2016**, *18*, 724–729. [CrossRef] [PubMed]
17. Ainane, A.; Abdoul-Latif, F.M.; Abdoul-Latif, T.M.; Ainane, T. Evaluation of biological activities of two essential oils as a safe environmental bioinsecticides: Case of Eucalyptus globulus and Rosmarinus officinalis. *Przegląd Nauk. Inżynieria I Kształtowanie Środowiska* **2020**, *29*, 544–556. [CrossRef]
18. Obame-Engonga, L.C.; Abdoul-Latif, F.M.; Ondo, J.P.; Sima-Obiang, C.; Ngoua-Meye-Misso, R.L.; Traoré, A. Phytochemical screening, antioxidant and antibacterial activities of Guibourtia ehie and Syzygium rowlandii medicinal plants from Gabon. *Int. J. Curr. Res.* **2017**, *9*, 56354–56360.
19. Abdoul-Latif, F.M.; Aouled Aden, S.; Abdoulkarim Omar, D.; Mohamed Abdoul-Latif, T.; Ainane, T. Bacterial infections in hemodialysis patients at peltier hospital, Djibouti. *Pharmacologyonline* **2021**, *3*, 877–882.
20. Elmi, A.; Spina, R.; Abdoul-Latif, F.; Yagi, S.; Fontanay, S.; Risler, A.; Duval, R.E.; Laurain-Mattar, D. Rapid screening for bioactive natural compounds in Indigofera caerulea Rox fruits. *Ind. Crop. Prod.* **2018**, *125*, 123–130. [CrossRef]
21. Elmi, A.; Abdoul-Latif, F.M.; Spina, R.; Dupire, F.; Philippot, S.; Marie-France, C.; Jacobs, H.; Laurain-Mattar, D. *Aloe djiboutiensis*: Antioxidant Activity, Molecular Networking-Based Approach and In Vivo Toxicity of This Endemic Species in Djibouti. *Molecules* **2021**, *26*, 3046. [CrossRef]
22. Abdoul-Latif, F.M.; Ainane, A.; Merito, A.; Ainane, T. Chemical composition and biological activities of essential oils from Djibouti. *J. Anal. Sci. Appl. Biotechnol.* **2022**, *4*, 1–9.
23. Ainane, A.; Abdoul-Latif, F.M.; Mohamed, J.; Attahar, W.; Ouassil, M.; Shybat, Z.L.; El Yaacoubi, A.; Ainane, T. Behaviour desorption study of the essential oil of Cedrus atlantica in a porous clay versus insecticidal activity against Sitophilus granarius: Explanation of the phenomenon by statistical studies. *Int. J. Metrol. Qual. Eng.* **2021**, *12*, 12. [CrossRef]
24. Attahar, W.; Mohamed Abdoul-Latif, F.; Mohamed, J.; Ainane, A.; Ainane, T. Antimicrobial and antioxidant activities of Trigonella foenum-graecum essential oil from the region of settat (Morocco). *Pharmacologyonline* **2021**, *2*, 434–442.
25. Susplugas, S.; Van Hung, N.; Bignon, J.; Thoison, O.; Kruczynski, A.; Sévenet, T.; Guéritte, F. Cytotoxic Arylnaphthalene Lignans from a Vietnamese Acanthaceae, *Justicia patentiflora*. *J. Nat. Prod.* **2005**, *68*, 734–738. [CrossRef] [PubMed]
26. Talbi, M.; Ainane, T.; Boriky, D.; Bennani, L.; Blaghen, M.; Elkouali, M. Antibacterial activity of Eudesmanolide compounds isolated from medicinal plant Artemisia herba-alba. *J. Mater. Environ. Sci.* **2015**, *6*, 2125–2128.
27. Ouassil, M.; Mohamed Abdoul-Latif, F.; Attahar, W.; Ainane, A.; Ainane, T. Chemical composition of bay laurel and rosemary essential oils from morocco and their antifungal activity against fusarium strains. *Pharmacologyonline* **2021**, *2*, 426–433.
28. Elansary, H.O.; Abdelgaleil, S.A.M.; Mahmoud, E.A.; Yessoufou, K.; Elhindi, K.; El-Hendawy, S. Effective antioxidant, antimicrobial and anticancer activities of essential oils of horticultural aromatic crops in northern Egypt. *BMC Complement. Altern. Med.* **2018**, *18*, 214. [CrossRef]
29. Mukarram, M.; Choudhary, S.; Khan, M.A.; Poltronieri, P.; Khan, M.M.A.; Ali, J.; Kurjak, D.; Shahid, M. Lemongrass Essential Oil Components with Antimicrobial and Anticancer Activities. *Antioxidants* **2021**, *11*, 20. [CrossRef]
30. AlMotwaa, S.M.; Al-Otaibi, W.A. Determination of the chemical composition and antioxidant, anticancer, and antibacterial properties of essential oil of Pulicaria crispa from Saudi Arabia. *J. Indian Chem. Soc.* **2022**, *99*, 100341. [CrossRef]
31. Shahi, A.K.; Tava, A. Essential Oil Composition of Three Cymbopogon Species of Indian Thar Desert. *J. Essent. Oil Res.* **1993**, *5*, 639–643. [CrossRef]
32. Ketoh, G.K.; Koumaglo, H.K.; Glitho, I.A. Inhibition of Callosobruchus maculatus (F.)(Coleoptera: Bruchidae) development with essential oil extracted from Cymbopogon schoenanthus L. Spreng.(Poaceae), and the wasp Dinarmus basalis (Rondani)(Hymenoptera: Pteromalidae). *J. Stored Prod. Res.* **2005**, *41*, 363–371. [CrossRef]
33. Katiki, L.; Chagas, A.; Takahira, R.; Juliani, H.; Ferreira, J.; Amarante, A. Evaluation of Cymbopogon schoenanthus essential oil in lambs experimentally infected with Haemonchus contortus. *Vet. Parasitol.* **2011**, *186*, 312–318. [CrossRef]
34. Malti, C.E.W.; El Haci, I.A.; Hassani, F.; Paoli, M.; Gibernau, M.; Tomi, F.; Casanova, J.; Bekhechi, C. Composition, Chemical Variability and Biological Activity of *Cymbopogon schoenanthus* Essential Oil from Central Algeria. *Chem. Biodivers.* **2020**, *17*, e2000138. [CrossRef] [PubMed]
35. Sawadogo, I.; Paré, A.; Kaboré, D.; Montet, D.; Durand, N.; Bouajila, J.; Bassolé, I.H.N. Antifungal and antiaflatoxinogenic effects of Cymbopogon citratus, Cymbopogon nardus, and Cymbopogon schoenanthus essential oils alone and in combination. *J. Fungi* **2022**, *8*, 117. [CrossRef]
36. Simiyu, S.K. Bioevaluation of Insecticidal and Repellen Plants from Central Region of Kenya and Chemical Identification of Bioactive Derivatives. Doctoral Dissertation, Kenyatta University, Nairobi, Kenyatta, 2004.
37. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226. [CrossRef]
38. Manosroi, J.; Dhumtanom, P.; Manosroi, A. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett.* **2006**, *235*, 114–120. [CrossRef]
39. Sharma, P.R.; Mondhe, D.M.; Muthiah, S.; Pal, H.C.; Shahi, A.K.; Saxena, A.K.; Qazi, G.N. Anticancer activity of an essential oil from Cymbopogon flexuosus. *Chem. Interact.* **2009**, *179*, 160–168. [CrossRef] [PubMed]

40. Yen, N.; Zainah, A.; Arapoc, D.J.; Mohamed ZA, M.A.; Shafii, K. Anticancer Effect and Apoptosis Induction of Cymbopogon citratus Plant on Head and Neck HTB43 Cancer Cell Lines. *Res. Dev. Semin. Bangi (Malays.)* **2016**, *48*, 48050355.
41. Sujatha, K.; Sirisha, K.B. Anti-cancer and Anti-oxidant activity of essential oils of *Rosmarinus officinalis*, *Azadirachta indica*, *Syzygium aromaticum* and *Cymbopogon nardus*. *J. Pharmacogn. Phytochem.* **2019**, *8*, 4493–4498.
42. Pan, D.; Machado, L.; Bica, C.G.; Machado, A.K.; Steffani, J.A.; Cadoná, F.C. In Vitro Evaluation of Antioxidant and Anticancer Activity of Lemongrass (*Cymbopogon citratus* (D.C.) Stapf). *Nutr. Cancer* **2021**, *74*, 1474–1488. [CrossRef] [PubMed]
43. Sharifi-Rad, J.; Ayatollahi, S.A.; Varoni, E.M.; Salehi, B.; Kobarfard, F.; Sharifi-Rad, M.; Sharifi-Rad, M. Chemical composition and functional properties of essential oils from Nepeta schiraziana Boiss. *Farmacia* **2017**, *65*, 802–812.
44. Tsuruoka, T.; Bekh-Ochir, D.; Kato, F.; Sanduin, S.; Shataryn, A.; Ayurzana, A.; Satou, T.; Li, W.; Koike, K. The essential oil of Mongolian *Nepeta sibirica*: A single component and its biological activities. *J. Essent. Oil Res.* **2012**, *24*, 555–559. [CrossRef]
45. Skorić, M.; Gligorijević, N.; Čavić, M.; Todorović, S.; Janković, R.; Ristić, M.; Radulović, S. Cytotoxic activity of Nepeta rtanjensis Diklić & Milojević essential oil and its mode of action. *Ind. Crops Prod.* **2017**, *100*, 163–170.
46. Jaradat, N.; Al-Maharik, N.; Abdallah, S.; Shawahna, R.; Mousa, A.; Qtishat, A. Nepeta curviflora essential oil: Phytochemical composition, antioxidant, anti-proliferative and anti-migratory efficacy against cervical cancer cells, and α-glucosidase, α-amylase and porcine pancreatic lipase inhibitory activities. *Ind. Crop. Prod.* **2020**, *158*, 112946. [CrossRef]
47. Ashrafi, B.; Rashidipour, M.; Gholami, E.; Sattari, E.; Marzban, A.; Kheirandish, F.; Khaksarian, M.; Taherikalani, M.; Soroush, S. Investigation of the phytochemicals and bioactivity potential of essential oil from Nepeta curvidens Boiss. & Balansa. *S. Afr. J. Bot.* **2020**, *135*, 109–116. [CrossRef]
48. Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines* **2016**, *3*, 25. [CrossRef]
49. Jamali, T.; Kavoosi, G.; Ardestani, S.K. In-vitro and in-vivo anti-breast cancer activity of OEO (Oliveria decumbens vent essential oil) through promoting the apoptosis and immunomodulatory effects. *J. Ethnopharmacol.* **2020**, *248*, 112313. [CrossRef] [PubMed]
50. Chung, K.-S.; Hong, J.Y.; Lee, J.-H.; Lee, H.-J.; Park, J.Y.; Choi, J.-H.; Park, H.-J.; Hong, J.; Lee, K.-T. β-Caryophyllene in the Essential Oil from Chrysanthemum Boreale Induces G1 Phase Cell Cycle Arrest in Human Lung Cancer Cells. *Molecules* **2019**, *24*, 3754. [CrossRef]
51. Piasecki, B.; Biernasiuk, A.; Skiba, A.; Skalicka-Woźniak, K.; Ludwiczuk, A. Composition, Anti-MRSA activity and toxicity of essential oils from Cymbopogon species. *Molecules* **2021**, *26*, 7542. [PubMed]
52. Sharma, A.; Nayik, G.A.; Cannoo, D.S. Pharmacology and Toxicology of Nepeta cataria (Catmint) Species of Genus Nepeta: A Review. *Plant Hum. Health* **2019**, *3*, 285–299. [CrossRef]