Antimicrobial Activity and Synergy Investigation of *Hypericum scabrum* Essential Oil with Antifungal Drugs

Layal Fahed 1,2, Marc El Beyrouthy 3,* Naïm Ouaini 3, Véronique Eparvier 1, Didier Stien 1,4, Sara Vitalini 5,6,7 and Marcello Iriti 5,6,7,8,*

**Abstract:** The chemical composition of Lebanese *Hypericum scabrum* essential oil (EO) was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Its antimicrobial activity was evaluated by determining its minimal inhibitory concentrations (MICs) against a Gram-negative and a Gram-positive bacterium, one yeast, and five dermatophytes. *H. scabrum* EO was most active on filamentous fungi (MIC values of 32–64 μg/mL). Synergy within the oil was investigated by testing each of the following major components on *Trichophyton rubrum*: α-pinene, limonene, myrcene, β-pinene and nonane, as well as a reconstructed EO. The antifungal activity of the natural oil could not be reached, meaning that its activity might be due, in part, to minor constituent(s). The interactions between *H. scabrum* EO and commercially available antifungals were assessed by the checkerboard test. A synergistic effect was revealed in the combination of the EO with amphotericin B.

**Keywords:** *Hypericum scabrum*; essential oil; chemical composition; antimicrobial activity; synergy

1. Introduction

Antimicrobial resistance is jeopardizing public health, and is thus turning into an urgent global concern. Resistance occurrence is the result of random genetic events, such as mutations and horizontal gene transfers that enable microbial genomes to evolve [1], while its persistence and spread in the ecosystem is favored by the selective pressure of antimicrobial agents [2,3].

One approach to avoid the emergence of resistant strains is combination therapy that consists of associating several antimicrobial agents. The chance of having mutants that are resistant to agents with independent mechanisms of action is extremely small. Resistance is thus less likely to develop [4]. Such associations could also be used to improve the therapy efficiency when they result in synergistic effects. Another advantage of such combinations is that lower doses are used [5], reducing the side effects and cost of the treatment [6]. In this field, associations between plant essential oils and classic antimicrobials have proven their efficiency [7,8].

---

**Article**

**Title:** Antimicrobial Activity and Synergy Investigation of *Hypericum scabrum* Essential Oil with Antifungal Drugs

**Authors:** Layal Fahed 1,2, Marc El Beyrouthy 3,* Naïm Ouaini 3, Véronique Eparvier 1, Didier Stien 1,4, Sara Vitalini 5,6,7 and Marcello Iriti 5,6,7,8,*

1. UPR 2301, Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette, France; layal.fahed@ul.edu.lb (L.F.); veronique.eparvier@cnrs.fr (V.E.); didier.stien@cnrs.fr (D.S.)
2. Natural Sciences Department, Faculty of Sciences II, Lebanese University, B.P. 90656, Fanar 1202, Lebanon
3. Department of Agricultural Sciences, Holy Spirit University of Kaslik, Jounieh 1200, Lebanon; naimouaini@usek.edu.lb
4. Laboratoire de Biodiversité et Biotechnologies Microbiennes (LBBM), Observatoire Oceanologique, UPMC Univ Paris 06, CNRS, Sorbonne Universités, 6650 Banyuls-sur-Mer, France
5. Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milan, Italy; sara.vitalini@unimi.it
6. Phytochem Laboratory, Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milan, Italy
7. National Interuniversity Consortium of Materials Science and Technology, 50121 Firenze, Italy
8. BAT Center-Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, Università degli Studi di Napoli Federico II, 80055 Portici, Italy
* Correspondence: marcelbeyrouthy@usek.edu.lb (M.E.B.); marcello.iriti@unimi.it (M.I.); Tel.: +961-3826419 (M.E.B.); +39-0250316766 (M.I.)

**Abstract:** The chemical composition of Lebanese *Hypericum scabrum* essential oil (EO) was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GG-MS). Its antimicrobial activity was evaluated by determining its minimal inhibitory concentrations (MICs) against a Gram-negative and a Gram-positive bacterium, one yeast, and five dermatophytes. *H. scabrum* EO was most active on filamentous fungi (MIC values of 32–64 μg/mL). Synergy within the oil was investigated by testing each of the following major components on *Trichophyton rubrum*: α-pinene, limonene, myrcene, β-pinene and nonane, as well as a reconstructed EO. The antifungal activity of the natural oil could not be reached, meaning that its activity might be due, in part, to minor constituent(s). The interactions between *H. scabrum* EO and commercially available antifungals were assessed by the checkerboard test. A synergistic effect was revealed in the combination of the EO with amphotericin B.

**Keywords:** *Hypericum scabrum*; essential oil; chemical composition; antimicrobial activity; synergy

---

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Citation:** Fahed, L.; Beyrouthy, M.E.; Ouaini, N.; Eparvier, V.; Stien, D.; Vitalini, S.; Iriti, M. Antimicrobial Activity and Synergy Investigation of *Hypericum scabrum* Essential Oil with Antifungal Drugs. *Molecules* **2021**, **26**, 6545. https://doi.org/10.3390/molecules26216545

---

**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
Plants belonging to the genus *Hypericum* are well known for their therapeutic benefits. The most popular species is *Hypericum perforatum* (St John’s Wort). Its extract is one of the best-selling herbal medicines in the world. It is used to treat mild-to-moderate depression [9,10].

*Hypericum scabrum* L. (Hypericaceae) has traditionally been, in different parts of the world, the basis of the medical treatment of many ailments, such as hemorrhoids [11], body heat [12], stomach ache, diarrhea [13], sun burn, and skin lesions [14].

Although extracts and essential oils (EOs) of *H. scabrum* have been investigated and reported to have relevant biological activities [15–18], our Lebanese EO differs significantly from those obtained elsewhere. It was, therefore, interesting to investigate its chemical composition and to evaluate its antimicrobial activity against selected microorganisms responsible for skin infections in humans. In fact, in the topical route of application that is often used traditionally, the EOs are administered in direct contact with the pathogen, and the adverse effects that they could cause when administered systemically become limited. In the present work, we demonstrate that the very good antifungal activity of the Lebanese *H. scabrum* does not originate from its major constituents, either alone or combined. Since the strong antifungal potential was due to minor components, we embarked upon investigating the potential of this oil to exacerbate the activity of conventional antimicrobials.

2. Results and Discussion

2.1. Chemical Analysis

The hydrodistillation of the aerial parts of *H. scabrum* yielded 0.6% essential oil. Thirty-seven compounds, representing 84.2% of the whole oil, were identified (Table 1; Figure 1).

![Pie chart representing the chemical composition of Hypericum scabrum essential oil (EO).](image1)

*α*-Pinene (Figure 2) was the most abundant compound (37.8%), followed by limonene (11.6%) (Figure 3), myrcene (5.6%) (Figure 4), *β*-pinene (3.4%) (Figure 5), and nonane (3%) (Figure 6). It is noteworthy that *α*-pinene was also the major compound of the previously studied *H. scabrum* essential oils from the following different regions of the world: Iran [19], Turkey [20], Tajikistan [21], and Uzbekistan [22]. This does not apply to the other major compounds, which were sometimes minorly present or even totally absent in these other samples.
Table 1. Chemical composition of *Hypericum scabrum* essential oil (EO).

| RI<sup>a</sup> | RI<sup>b</sup> | Compounds | CAS No | Molecular Formula | (%) |
|----------------|----------------|-----------|--------|-------------------|-----|
| 901            | -              | Nonane    | 111-84-2 | C<sub>9</sub>H<sub>20</sub> | 3.0 |
| 938            | 1076           | α-Pinene  | 80-56-8 | C<sub>10</sub>H<sub>16</sub> | 37.8|
| 980            | 1118           | β-Pinene  | 127-91-3 | C<sub>10</sub>H<sub>16</sub> | 3.4 |
| 993            | 1174           | Myrcene   | 123-35-3 | C<sub>10</sub>H<sub>16</sub> | 5.6 |
| 1030           | 1203           | Limonene  | 138-86-3 | C<sub>10</sub>H<sub>16</sub> | 11.6|
| 1045           | 1269           | cis-β-Ocimene | 3338-35-4 | C<sub>10</sub>H<sub>16</sub> | 1.1 |
| 1050           | 1253           | trans-β-Ocimene | 3779-61-1 | C<sub>10</sub>H<sub>16</sub> | 0.9 |
| 1100           | 1490           | Undecane  | 1120-21-4 | C<sub>11</sub>H<sub>24</sub> | 1.6 |
| 1134           | 1493           | allo-Ocimene | 673-84-7 | C<sub>10</sub>H<sub>16</sub>O | 1.7 |
| 1138           | 1664           | Pinocarveol | 5947-36-4 | C<sub>10</sub>H<sub>16</sub>O | 0.1 |
| 1152           | 1683           | trans-Verbénol | 1820-09-3 | C<sub>10</sub>H<sub>16</sub>O | 0.2 |
| 1168           | 1697           | α-Phellandren-8-ol | 1686-20-0 | C<sub>10</sub>H<sub>18</sub>O | 0.1 |
| 1189           | 1706           | a-Terpineol | 98-55-5 | C<sub>10</sub>H<sub>18</sub>O | 0.1 |
| 1196           | 1804           | Myrtenol  | 515-00-4 | C<sub>10</sub>H<sub>16</sub>O | 0.1 |
| 1352           | 1466           | a-Cubebene | 17699-14-8 | C<sub>13</sub>H<sub>24</sub> | 0.1 |
| 1377           | 1497           | a-Copaene | 3856-25-5 | C<sub>13</sub>H<sub>24</sub> | 0.3 |
| 1385           | 1533           | β-Bourbonene | 5208-59-3 | C<sub>13</sub>H<sub>24</sub> | 0.1 |
| 1415           | 1612           | trans-Caryophyllene | 87-44-5 | C<sub>13</sub>H<sub>24</sub> | 1.3 |
| 1437           | 1628           | Aromadendrene | 109119-91-7 | C<sub>13</sub>H<sub>24</sub> | 0.3 |
| 1439           | 1650           | a-Guaiene | 3691-12-1 | C<sub>13</sub>H<sub>24</sub> | 0.3 |
| 1455           | 1689           | trans-β-Farnesene | 18794-84-8 | C<sub>13</sub>H<sub>24</sub> | 2.0 |
| 1477           | 1726           | Germacrene D | 23986-74-5 | C<sub>13</sub>H<sub>24</sub> | 1.3 |
| 1485           | 1711           | β-Seinen | 17066-67-0 | C<sub>13</sub>H<sub>24</sub> | 0.4 |
| 1495           | 1740           | Valencene | 4630-07-3 | C<sub>13</sub>H<sub>24</sub> | 1.2 |
| 1500           | 1740           | a-Murolene | 10208-80-7 | C<sub>13</sub>H<sub>24</sub> | 0.4 |
| 1515           | 1776           | γ-Cadinene | 39029-41-9 | C<sub>13</sub>H<sub>24</sub> | 0.7 |
| 1525           | 1772           | δ-Cadinene | 483-76-1 | C<sub>13</sub>H<sub>24</sub> | 1.3 |
| 1526           | 1773           | a-Cadinene | 24406-05-1 | C<sub>13</sub>H<sub>24</sub> | 0.2 |
| 1541           | 1918           | a-Calacorene | 38599-17-6 | C<sub>13</sub>H<sub>24</sub>O | 0.2 |
| 1566           | 2050           | Nerolidol | 142-50-7 | C<sub>15</sub>H<sub>30</sub>O | 0.4 |
| 1577           | 2088           | Spathulenol | 6750-80-3 | C<sub>15</sub>H<sub>30</sub>O | 2.7 |
| 1585           | 2098           | Globulol  | 489-41-8 | C<sub>15</sub>H<sub>30</sub>O | 0.5 |
| 1591           | 2104           | Viridiflorol | 552-02-3 | C<sub>15</sub>H<sub>30</sub>O | 0.3 |
| 1640           | 2188           | T-Cadinol | 5937-11-1 | C<sub>15</sub>H<sub>30</sub>O | 0.9 |
| 1645           | 2145           | Torreyol  | 19435-97-3 | C<sub>15</sub>H<sub>30</sub>O | 0.3 |
| 1649           | 2188           | α-Eudesmol | 473-16-5 | C<sub>15</sub>H<sub>30</sub>O | 0.2 |
| 1650           | 2256           | α-Cadinol | 481-34-5 | C<sub>15</sub>H<sub>30</sub>O | 1.4 |
|                |                | Total identified | 84.2 |

<sup>a</sup> Retention index on a DB-5MS column. <sup>b</sup> Retention index on an HP Innowax column.

Figure 2. Chemical structure of α-pinene.

Figure 3. Chemical structure of limonene.
2.2. Antimicrobial Activity

The results of the antimicrobial activity of *H. scabrum* EO are presented in Table 2. EOs with minimal inhibitory concentrations (MICs) of 128 μg/mL and below are generally considered active [23,24].

| Pathogens       | S. aureus ATCC 29213 | C. albicans ATCC 10231 | P. aeruginosa CIP 82118 | T. rubrum SNB-TR1 | T. mentagrophytes SNB-TM1 | T. soudanense SNB-TS1 | T. violaceum SNB-TV1 | T. tonsurans SNB-TT1 |
|-----------------|----------------------|------------------------|--------------------------|-------------------|---------------------------|-----------------------|--------------------|--------------------|
| *Hypericum scabrum* EO                  | > 512                | 512                    | > 512                     | 64                | 32                        | 32                    | 32                 | 64                 |
| Reference compounds | 1<sup>a</sup>      | 1<sup>b</sup>          | 0.25<sup>c</sup>           | 2<sup>b</sup>          | 1<sup>d</sup>           | 2<sup>b</sup>            | 2<sup>b</sup>        | 4<sup>b</sup>        |

<sup>a</sup> Oxacillin; <sup>b</sup> fluconazole; <sup>c</sup> gentamicin; <sup>d</sup> itraconazole.

The oil was inactive on *Pseudomonas aeruginosa* and *Staphylococcus aureus* (MIC > 512 μg/mL). Conversely, *H. scabrum* EO presented significant antifungal activity against filamentous dermatophytic fungi (MIC values of 32–64 μg/mL, whereas *Candida albicans* was weakly sensitive (MIC of 512 μg/mL).

Dermatophytes were the most sensitive microorganisms, which is a result that can be explained by the functional role of volatile organic compounds (VOCs), since the majority of pathogens infecting plants are fungi, most of them being filamentous [25,26].

In an attempt to shed light on the origin of the antifungal activity, we individually tested the major components and a reconstructed EO against *Trichophyton rubrum* (Table 3). The artificial EO was prepared using the major compounds in pure form, in the same proportions as in the EO, whereas the minor compounds were replaced by DMSO (38.6%).
Table 3. Antifungal activity (MIC in µg/mL) of the major constituents of *Hypericum scabrum* EO and a reconstructed EO from the major constituents on *Trichophyton rubrum*.

|           | Nonane | α-Pinene | β-Pinene | Myrcene | Limonene | Reconstructed EO (61.4%) |
|-----------|--------|----------|----------|---------|----------|-------------------------|
| *T. rubrum* SNB-TR1 | 512    | 512      | 512      | 512     | 512      | 256                     |

α-Pinene, limonene, myrcene, β-pinene, and nonane showed no significant activity (MIC of 512 µg/mL). The reconstructed EO exhibited moderate activity (256 µg/mL), which points to a probable synergy between the major compounds. It is, however, less active than the natural EO (64 µg/mL), indicating that minor constituents contribute to the overall activity of the EO.

The interaction between *H. scabrum* and antifungal drugs was tested by the checkerboard assay on *T. rubrum*, and the results are given in Table 4. A synergistic effect (FICI 0.5) was observed by combining the EO with amphotericin B. When the EO was added at a concentration of 32 µg/mL, the MIC of amphotericin B was lowered from 4 to 0.125 µg/mL, thus the combined use of *Hypericum scabrum* essential oil with amphotericin B could reduce the amount of amphotericin B by 32 for the same antifungal activity. On the other hand, the associations with fluconazole and griseofulvin were moderately synergistic (FICI 0.6).

Table 4. Combined effects of *Hypericum scabrum* essential oil and conventional antimicrobial drugs on *Trichophyton rubrum*.

| Combination | EO: Fluconazole | EO: Griseofulvin | EO: Amphotericin B |
|-------------|-----------------|-----------------|-------------------|
| EO          | MICₐ            | MICₐ            | MICₐ              |
|             | 64              | 64              | 64                |
|             | MICₜ            | MICₜ            | MICₜ              |
|             | 32              | 32              | 32                |
|             | FIC             | FIC             | FIC               |
|             | 0.5             | 0.5             | 0.5               |
| Drug        | MICₐ            | MICₜ            | MICₗ              |
|             | 2               | 0.0625          | 0.125             |
|             | FIC             | FIC             | FIC               |
|             | 0.6             | 0.6             | 0.5               |

MICₐ: MIC of the EO or the drug alone (in µg/mL); MICₜ: MIC of the EO or the drug at the relative proportion displaying the highest synergy (in µg/mL); FIC: fractional inhibitory concentration; FICI: FIC index.

3. Materials and Methods

3.1. Plant Material

Aerial parts of *Hypericum scabrum* were collected in June 2013 from Mzaar Kferdebian, Mount Lebanon (33°59'38" N 35°50'5" E) at an altitude of 1818 m. The plant identification was based on 'Nouvelle flore du Liban et de la Syrie' by Paul Mouterde [27]. Air drying of the plant materiel was performed in a shady place for two weeks at room temperature.

3.2. Essential Oil Extraction

The extraction of the essential oil was carried out by hydrodistillation for three hours using a Clevenger-type apparatus according to the European Pharmacopoeia, 1997 [28].

3.3. GC Analyses

Analytical gas chromatography was performed on a Thermo Electron Corporation gas chromatograph fitted with a flame ionization detector (FID), a DB-5 MS capillary column (30 m × 0.25 mm) with 0.1 µm film thickness or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 µm). Helium was the carrier gas (0.7 mL/min). The column temperature was initially set to 35 °C before being gradually increased to 85 °C at 5 °C/min, held for 20 min at 85 °C, raised to 300 °C at 10 °C/min and finally held for 5 min at 300 °C. Diluted 1 µL samples (1/100, v/v) were injected at 250 °C manually and in the splitless mode. Flame ionization detection (FID) was performed at 310 °C.
3.4. GC–MS Analyses

The GC–MS analyses were performed using an Agilent 6890 gas chromatograph coupled with 5975 mass detector. The 7683 B auto sampler injected 1 µL of each diluted oil sample (1/100, v/v). A fused silica capillary column DB-5 MS (30 m × 0.25 mm internal diameter, film thickness 0.1 µm) or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 µm) was used. Helium was the carrier gas (0.7 mL/min). The oven temperature program was identical to that described above (c.f. 3.3 GC Analysis). The mass spectra were recorded at 70 eV with an ion source temperature of 310 ºC and a transfer line heated to 320 ºC. The acquisition was recorded in full scan mode (50–400 m/z).

3.5. Qualitative and Quantitative Analysis

Most constituents were identified by gas chromatography by comparing their retention indices (RI) with those from the literature [29,30] or with those of authentic compounds obtained from Sigma-Aldrich (Beirut-Lebanon, and Paris-France). The retention indices were determined relatively to a homologous series of n-alkanes (C₈ to C₂₄) analyzed under the same operating conditions. Concomitantly, their mass spectra on both columns were compared with those provided in the NIST and Wiley 275 libraries, which are our homemade libraries constructed with pure compounds and EOs of known composition or with mass spectra from the literature [29,31]. The relative concentration of each component was calculated based on the GC peak areas without using correction factors.

3.6. Antimicrobial Activity

Gram-negative bacterial strain *Pseudomonas aeruginosa* CIP 82118, Gram-positive bacterial strain *Staphylococcus aureus* ATCC 29213, yeast *Candida albicans* ATCC 10231, and the clinical isolates of dermatophytes *Trichophyton rubrum* SNB-TR1, *Trichophyton mentagrophytes* SNB-TM1, *Trichophyton soudanense* SNB-TS1, *Trichophyton violaceum* SNB-TV1 and *Trichophyton tonsurans* SNB-TT1 [32] were used in this study.

The antimicrobial activity of the EOs was measured using a broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [33–37]. The essential oil and its major components were diluted in DMSO and were tested at concentrations ranging from 16 to 512 µg/mL. The microplates were incubated at 37 ºC for 24 h for bacteria, 48 h for yeasts, and 5 days for dermatophytes. The minimal inhibitory concentrations (MIC) refer to the lowest concentrations that prevent visible microbial growth (Tables 2 and 3). Oxacillin and gentamicin (0.03–16 µg/mL) were used as reference antibiotics, while itraconazole (0.03–16 µg/mL) and fluconazole (0.125–64 µg/mL) were used as positive controls for the antifungal assays. The antimicrobial standards were purchased from Molekula–Dorset, UK and the pure terpenes from Sigma-Aldrich, France.

3.7. Synergy Test by Microdilution Checkerboard Technique

This test is performed in a 96-well microplate and is based on the microdilution method. In particular, two-fold dilution series of the essential oil are made in the vertical direction on the plate while those of the antimicrobial agent are made in the horizontal direction of the same plate, in order to have a fixed amount of the first agent in each row and column and a decreasing amount of the second agent [38,39]. The essential oil was tested at concentrations ranging from 256 to 8 µg/mL. Antimicrobial agents were tested at concentrations ranging from 16 to 0.03 µg/mL for griseofulvin and amphotericin B, and from 64 to 0.125 µg/mL for fluconazole.

The quantitative assessment of the interactions was based on the calculation of fractional inhibitory concentrations (FICs) defined as the ratio of the MIC of the combinations of the two components to the MIC of the essential oil or the drug alone, and the FIC index (FICI), which is the sum of the two FICs. The results were interpreted as follows:
4. Conclusions

Our results showed that H. scabrum EO exhibited interesting antifungal activity. This activity could not be attributed to one major constituent of the oil. It clearly resulted from the interaction of several constituents, including minor ones. In addition, its synergistic interaction with amphotericin B, and the moderately synergistic association with fluconazole and griseofulvin, suggested that combinations between this EO and conventional antifungal agents may lead to new, more potent formulations with less dose-related toxicity.

Author Contributions: Conceptualization, D.S., M.E.B. and L.F.; methodology, L.F., S.V. and M.I.; software, L.F.; formal analysis, L.F.; investigation, L.F., S.V. and M.I.; data curation, L.F.; writing—original draft preparation, L.F.; writing—review and editing, D.S., M.E.B. and L.F.; supervision, D.S., M.E.B., N.O., M.I. and V.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agence Nationale de la Recherche, grant number CEBa, ref. 165 ANR-10-LABX-25-01 and benefitted from the support of CNRS Lebanon.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Dobrnndt, U.; Hentschel, U.; Kaper, J.B.; Hacker, J. Genome plasticity in pathogenic and nonpathogenic enterobacteria. *Curr. Top. Microbiol. Immunol.* 2002, 264, 157–175.

2. Duval-Iflah, Y.; Rmbaud, P.; Tancrede, C.; Rousseau, M. R-plasmid transfer from Serratia liquefaciens to *Escherichia coli* in vitro and in vivo in the digestive tract of gnotobiotic mice associated with human fecal flora. *Infect. Immun.* 1980, 28, 981–990. [CrossRef] [PubMed]

3. Andremont, A. Comment définir le potentiel de sélection de la résistance bactérienne aux antibiotiques? *Med. Mal. Infect.* 2005, 35, 207–211. [CrossRef]

4. Fischbach, M.A. Combination therapies for combatting antimicrobial resistance. *Curr. Opin. Microbiol.* 2011, 14, 519–523. [CrossRef] [PubMed]

5. Williamson, E.M. Synergy and other interactions in phytomedicines. *Phytotherapy* 2001, 8, 401–409. [CrossRef]

6. Fadli, M.; Saad, A.; Sayadi, S.; Chevalier, J.; Mezrioui, N.E.; Pagès, J.M.; Hassani, L. Antibacterial activity of Thymus maroccanus and Thymus broussonetii essential oils against nosocomial infection—bacteria and their synergistic potential with antibiotics. *Phytotherapy* 2012, 19, 464–471. [CrossRef]

7. Mahboubi, M.; Bidgoli, F.G. In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of *Candida albicans*. *Phytomedicine* 2010, 17, 771–774. [CrossRef]

8. Rosato, A.; Vitali, C.; De Laurentis, N.; Armenise, D.; Mililio, M. Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytotherapy* 2007, 14, 727–732. [CrossRef]

9. Cavaliere, C.; Rea, P.; Lynch, M.D.; Blumenthal, M. Herbal supplement sales experience slight increase in 2008. *HerbalGram* 2009, 82, 58–61.

10. Gambarioglu, U.; Turkoglu, I. An ethnobotanical survey of medicinal plants in Sivrice (Elazığ-Turkey). *J. Ethnopharmacol.* 2004, 92, 197–207. [CrossRef]

11. Cakicioglu, U.; Turkoglu, I. An ethnobotanical survey of medicinal plants in Sivrice (Elazığ-Turkey). *J. Ethnopharmacol.* 2004, 92, 197–207. [CrossRef] [PubMed]

12. Amiri, M.S.; Jabbarzadeh, P.; Akhondi, M. An ethnobotanical survey of medicinal plants used by indigenous people in Zangelanlo district, Northeast Iran. *J. Med. Plant Res.* 2012, 6, 749–753.

13. Sezik, E.; Yesiltada, E.; Shadiroyatov, H.; Kullive, Z.; Nigmatullaev, A.M.; Aripov, H.N.; Takaishi, Y.; Takeda, Y.; Honda, G. Folk medicine in Uzbekistan: I. Toshkent, Dijizzax, and Samarqand provinces. *J. Ethnopharmacol.* 2004, 92, 197–207. [CrossRef]

14. Fakir, H.; Korkmaz, M.; Güller, B. Medicinal Plant Diversity of Western Mediterranean Region in Turkey. *J. Appl. Biol. Sci.* 2009, 3, 3–4.
