ANTIMICROBIAL ACTIVITY OF NATIVE AND EXOTIC PLANT SPECIES AGAINST THE CAUSATIVE AGENTS OF LOWER RESPIRATORY TRACT INFECTIONS; INTERACTIONS WITH CONVENTIONAL ANTIBIOTICS

PhD THESIS ABSTRACT

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The doctoral thesis includes:

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This summary shows selective references and iconography, respecting numbering and content of the thesis.

**Key words**: essential oils, sinergism, antibacterial action, lower respiratory tract infections, plant extracts.
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ANNEX 1. Published papers on dissertation topic
JUSTIFICATION FOR CHOOSING THE DISSERTATION TOPIC, AIM AND OBJECTIVES

The World Health Organization estimates that daily approximately 50,000 people die of infectious diseases, these diseases being responsible for one third of deaths worldwide. The major cause of the high mortality rate from infectious diseases is microbial resistance to antibiotics. Irrational prescribing and unjustified use or abuse of antibiotics have favored the emergence of antibiotic-resistant microbial strains.

Bacterial strains that are resistant to many antibiotics (multi-drug-resistant strains) are a major risk to global health security (180).

It is estimated that 90-95% of Staphylococcus aureus strains are resistant to penicillin, while in Asian countries 70-80% of the strains are resistant to methicillin (180, 181).

If in 2009 some European countries (Portugal, Romania) reported that 25-50% of Staphylococcus aureus strains isolated from patients with nosocomial infections were methicillin-resistant, in 2011 the same countries reported rates of 50% for the same strains.

In recent years, there is a worrying increase in carbapenem-resistant microorganisms. In 2009, carbapenem resistance was a rare phenomenon in most European countries (less than 1% for Klebsiella pneumoniae). In only three years, the percentage increased to over 50% in many European countries (Portugal, Greece, Romania). In addition, carbapenem-resistant Escherichia coli and Acinetobacter spp. strains have also been identified (182).

The emergence of antibiotic-resistant microbial strain is an explosive phenomenon. Unfortunately, the speed at which microbial resistance occurs is higher than the rate at which new effective antimicrobial agents become available. In these circumstances, the identification of high-efficiency, good-tolerability new antibacterial substances is a current priority in therapeutics (182).

An alternative in the treatment of antibiotic resistance is represented by medicinal herbs. The literature mentions the antibacterial effect of plant extracts and substances, as well as the synergistic effects of certain plant extract combinations against various pathogenic microorganisms. Moreover, some plant extracts and substances act synergistically with antibiotics against multi-drug resistant microorganisms. Antibiotic-plant extract/substance combinations with synergistic action are very promising.
in the treatment of infections caused by multi-drug resistant pathogens. Such combinations have a wider antimicrobial spectrum, stronger effects and less toxicity than either of the components and, in addition, prevent the emergence of resistant mutants (180).

The substances of plant origin with antibacterial effects (polyphenols, terpenoids) are less active than antibiotics. However, these substances provide plant resistance to infection, which can be accounted for only by some synergistic effects. In this respect, the example of berberine and 5-methoxy hydrocarpin is very conclusive. Both substances are produced by Berberis vulgaris L. (Berberidaceae, barberry). The effectiveness of berberine as antimicrobial agent is much reduced against bacteria expressing MDR efflux pumps (multi-drug resistance) which significantly reduce berberine accumulation of in the bacterial cell. Barberry also produces 5-methoxy hydrocarpin which has the ability to block the MDR efflux pumps. Thus, berberine-5-methoxy hydrocarpin combination is a highly effective antimicrobial agent (181).

Many antibiotic-plant extract/substance combinations were found to have synergistic effects.

Essential oils from Rosmarinus officinalis L. (Lamiaceae, rosemary), Coriandrum sativum L. (Apiaceae, coriander), Micromeria fruticosa L. (Lamiaceae), Mentha piperita L. (Lamiaceae, mint), Cumium cymnum L. (Apiaceae) act synergistically with gentamicin, cephalothin, ceftriaxone and nystatin against a wide variety of microorganisms including Bacillus megaterium NRS, Bacillus brevis FMC3, Enterococcus faecalis ATCC 15753, Staphylococcus aureus Cowan 1 and Streptococcus faecalis DC 74 (180, 183).

Synergistic effects were also found for ethanolic extracts from Rhus coriaria L. (Anacardiceae, cashew) and Sacropoterium spinosum L. (Rosaceae) seeds, and Rosa damascena Mill. (Rosaceae, Damask rose) flowers in combination with oxytetracycline, penicillin G, cephalaxin, sulfadimethoxin, enrofloxacin against multi-drug-resistant Pseudomonas aeruginosa strains and clinical isolates of methicillin-resistant Staphylococcus aureus.

Synergistic effects were also found for the following combinations: erythromycin-methanol extract from Euphorbia hirta L. (Euphorbiaceae) leaves against clinical isolates of Staphylococcus aureus, tetracycline-methanolic extract from Tectona grandis L. (Verbenaceae) against Serratia marcescens and Pseudomonas aeruginosa, oxytetracycline-methanolic extract of Thespesia populnea L. (Malvaceae, Pacific rose), ciprofloxacin-extract of Angelica sinensis and Melissa officinalis against Enterobacteriaceae and Pseudomonas aeruginosa (180, 183).
When some antibiotics (penicillin sodium salt, amoxicillin, chloramphenicol, oxytetracycline, erythromycin, ciprofloxacin) are combined with various extracts from *Helichrysum longifolium* D.C. (*Asteraceae*), synergistic (65%), antagonistic (6.67%) and indifferent effects (28.33%) against standard strains and clinical isolates of *Pseudomonas aeruginosa* ATCC1 9582, *Staphylococcus aureus* ATCC 6538, *Salmonella* spp, *Bacillus cereus* ATCC 10702, *Proteus vulgaris* ATCC 6830, and *Staphylococcus aureus* OKOH1 were noticed.

For some substances of plant origin the mechanism of synergistic action with different antibiotics has been elucidated. Epigallocatechin gallate from *Camellia sinensis* L. Kuntze, baicalin from *Scutellaria amoena* CH Wright, corillagin from *Arctostaphylos uva-ursi* L. Spreng, tellimagrandin I and rugosin B from *Rosa canina* L. act synergistically with beta-lactam antibiotics. For instance, corilagin inhibits the expression and activation of penicillin binding protein 2a (PBP2) for which beta-lactam antibiotics have a very low affinity. Epigallocatechin gallate inhibits the activity of penicillinase produced by *Staphylococcus aureus*, thus increasing its susceptibility to penicillin. Many flavone glycosides inhibit the activity of topoisomerase IV, while allicin from *Allium sativum* L. reduces RNA synthesis.

Some substances of plant origin (carnosic acid from *Rosmarinus officinalis* L. isopimaran-type diterpenes of *Lycopus europaeus* L., epigallocatechin gallate, isoflavones isolated from *Lupinus argentes* Purish inhibit the activity of efflux pumps thereby increasing the antibiotic concentration in bacterial cell. By the same mechanism, reserpine potentiates the activity of fluoroquinolones and tetracycline against methicillin-resistant *Staphylococcus aureus* strains (181).

Carnosol acts synergistically with aminoglycosides against vancomycin-resistant enterococci. Both kaempferol and quercetin act synergistically with clindamycin against antibiotic-resistant *Propionibacterium acnes* strains. Gerianol isolated from *Helichrysum italicumare* increases the effectiveness of beta-lactam antibiotics against multi-drug-resistant strains of *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenesare, Acinetobacter baumannii*, the sensitivity of the latter to novobiocin increasing significantly in the presence of ellagic and tannic acids (183).

The PhD thesis aimed to evaluate the antimicrobial activity of some native and exotic plant species against infectious agents of the lower respiratory tract and to study the interactions occurring when these are combined with conventional antibiotics.
Plant species known from the literature to have antimicrobial effects, and which interact with the studied antibiotics against reference bacterial strains were selected. The study primarily aimed to assess the antibacterial action against antibiotic-resistant strains isolated from patients with lower respiratory tract infections.

The main **objectives** of the thesis were:
- to obtain plant extracts and characterize them physico-chemically;
- to assess their antibacterial potential against reference strains and antibiotic-resistant strains isolated from patients with lower respiratory tract infections;
- to study the interactions occurring when these extracts are combined with conventional antibiotics.
8.1. Trachyspermum ammi L. Sprague-general data

*Trachyspermum ammi* L. Sprague (syn. *Ammi copticum* L., *Carum copticum* (L.) Link, *Trachyspermum coticum* Link, ajowan, ajwain, Indian cumin) is an annual aromatic herb, that belong to the family *Apiaceae*, order *Apiales* class *Magnoliatae* (*Dicotyledonatae*) phylum *Magnoliophyta* (*Angyospermatophyta*) (199).

**Fig.8.1.** *Trachyspermum ammi* L. Sprague

**Fig.8.2.** Leaves of *Trachyspermum ammi* L. Sprague

8.2. Physico-chemical characterization

By steam distillation of ajowan fruits a light-yellow essential oil with a characteristic odor was obtained; it was dried over anhydrous sodium sulfate and stored at 4°C. Yield was 7.10±0.26% (ml essential oil/100 g plant product).

8.2.1. Determination of relative density

The isolated essential oil had a relative density of 0.94±0.00.

8.2.2. GC-MS and GC-FID study of chemical composition

In tab.8.1. are listed the compounds identified in the essential oil of *Trachyspermi fructus*, in order of their elution on DB-5MS column, retention indices, and their percentage concentrations. The 16 compounds identified in the essential oil accounted for 98.93% of the total percentage. Quantitatively, thymol was the predominant component (50.75%), followed by γ-terpinene (25.94%), p-cymene (18.31%) and β-pinene (2.27%).

---

### TABLE 8.1.
Chemical composition of the essential oil of *Trachyspermi fructus*

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>IR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Percentage concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>α-tuinen</td>
<td>922</td>
<td>924</td>
<td>0.41</td>
</tr>
<tr>
<td>2.</td>
<td>α-pinene</td>
<td>928</td>
<td>933</td>
<td>0.21</td>
</tr>
<tr>
<td>3.</td>
<td>camphene</td>
<td>943</td>
<td>946</td>
<td>0.01</td>
</tr>
<tr>
<td>4.</td>
<td>β-pinene</td>
<td>974</td>
<td>974</td>
<td>2.27</td>
</tr>
<tr>
<td>5.</td>
<td>myrcene</td>
<td>987</td>
<td>988</td>
<td>0.49</td>
</tr>
<tr>
<td>6.</td>
<td>Δ-3-caren</td>
<td>1004</td>
<td>1008</td>
<td>0.02</td>
</tr>
<tr>
<td>7.</td>
<td>α-terpinene</td>
<td>1013</td>
<td>1014</td>
<td>0.28</td>
</tr>
<tr>
<td>8.</td>
<td>p-cymene</td>
<td>1029</td>
<td>1030</td>
<td>18.31</td>
</tr>
<tr>
<td>9.</td>
<td>γ-terpinene</td>
<td>1067</td>
<td>1064</td>
<td>25.94</td>
</tr>
<tr>
<td>10.</td>
<td>α-terpinolene</td>
<td>1082</td>
<td>1084</td>
<td>0.06</td>
</tr>
<tr>
<td>11.</td>
<td>terpinen-4-ol</td>
<td>1174</td>
<td>1174</td>
<td>0.10</td>
</tr>
<tr>
<td>12.</td>
<td>trans-anethole</td>
<td>1280</td>
<td>1282</td>
<td>0.03</td>
</tr>
<tr>
<td>13.</td>
<td>thymol</td>
<td>1296</td>
<td>1295</td>
<td>50.75</td>
</tr>
<tr>
<td>14.</td>
<td>piperitone</td>
<td>1339</td>
<td>1340</td>
<td>0.01</td>
</tr>
<tr>
<td>15.</td>
<td>eugenol</td>
<td>1351</td>
<td>1356</td>
<td>0.03</td>
</tr>
<tr>
<td>16.</td>
<td>β-caryophyllene</td>
<td>1409</td>
<td>1411</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>98.93</strong></td>
</tr>
</tbody>
</table>

Kovats indices calculated on DB-5MS capillary column;
Kovats indices listed in the literature (209, 225)

---

**Fig. 8.3.** GC-MS chromatogram of essential oil from *Trachyspermi fructus*
8.4. Study of antibacterial activity by broth dilution method

Regarding the clinical isolates of *Staphylococcus aureus*, thymol had a high MIC value (8 mg/ml) against both tested strains, result consistent with the diffusimetric antibiogram. Ajowan essential oil was more active than thymol against both strains (0.125 vs. 8 mg/ml) (tab.8.2.).

As also shown by diffusimetric antibiogram, all tested clinical isolates of *Streptococcus pneumoniae* were susceptible to ajowan essential oil (MIC=0.06-0.5 mg/ml) and thymol (MIC=0.125-1 mg/ml) (tab.8.2.).

The least sensitive strain, both to the action of ajowan essential oil and thymol was *Pseudomonas aeruginosa* 2351. Thymol showed lower activity than ajowan essential oil (16 vs. 8 mg/ml).

The other Gram-negative bacteria, *Moraxella catarrhalis*, was very sensitive to the activity of ajowan essential oil and thymol (tab.8.2.).

**TABLE 8.2.**

MIC and MBC values of essential oil from *Trachyspermi fructus* and thymol

<table>
<thead>
<tr>
<th>Tested microorganism</th>
<th>Ajw MIC*</th>
<th>Ajw MBC*</th>
<th>Tim MIC*</th>
<th>Tim MBC*</th>
<th>Ax MIC**</th>
<th>Cip MIC**</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> 37</td>
<td>0.125</td>
<td>0.125</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> 4185</td>
<td>0.125</td>
<td>0.125</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> 4409</td>
<td>0.06</td>
<td>0.125</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> 4566</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> 4546</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>0.5</td>
<td>0.03</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> 4732</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> 4423</td>
<td>0.06</td>
<td>0.06</td>
<td>0.125</td>
<td>0.125</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> 2351</td>
<td>8</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em> 2002</td>
<td>0.125</td>
<td>0.125</td>
<td>0.5</td>
<td>0.5</td>
<td>32</td>
<td>0.03</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em> 4708</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>32</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*=mg/ml; **=µg/ml; Ajw=ajowan essential oil, Tim=thymol, Ax=amoxicillin, Cip=ciprofloxacine
8.5. Study of the interactions of *Trachyspermi fructus* essential oil and thymol with amoxicillin by checkboard method

The effects of ajowan essential oil/thymol (Ajw/Tim) - amoxicillin (Ax) combinations were tested against the following clinical isolates: *Staphylococcus aureus* 37 and 4185, *Streptococcus pneumoniae* 4409, 4566, 4546, 4732 and 4423, *Moraxella catarrhalis* 2002 and 4708 and *Pseudomonas aeruginosa* 2351.

The effects of Ajw/Tim-Ax combinations ranged from synergy to indifference. No combination had antagonistic effect.

For *Staphylococcus aureus* 37 strain, ajowan essential oil-amoxicillin combination had a synergistic effect at three different mixing ratios:

- 0.015 mg/ml Ajw – 0.5 µg/ml Ax (FICI=0.37);
- 0.03 mg/ml Ajw – 0.5 µg/ml Ax (FICI=0.49);
- 0.03 mg/ml Ajw – 0.25 µg/ml Ax (FICI=0.36) (fig.8.5.a).

For the same species, the effects of Tim-Ax combination ranged from synergy to indifference depending on the mixing ratios. The following combinations deserve mention:

- 2 mg/ml Tim – 0.5 µg/ml Ax – synergistic effect (FICI=0.5);
- 4 mg/ml Tim – 0.5 µg/ml Ax – additive effect (FICI=0.75);
- 4 mg/ml Tim – 0.25 µg/ml Ax – additive effect (FICI=0.62) (fig.8.5.b).

The other combinations were indifferent (tab.8.3.).

The combination 1 mg/ml Tim - 1 mg/ml Ax had synergistic effect against *Staphylococcus aureus* 4185 (FICI=0.37) (tab.8.3.). Two combinations had additive effect:

- 4 mg/ml Tim – 1 µg/ml Ax (FICI=0.75);
- 4 mg/ml Tim – 0.5 µg/ml Ax (FICI=0.62) (fig.8.5.d).

The other thymol-amoxicillin combinations were indifferent, FICI ranging from 1.03 to 1.12 (tab.8.3.). Against the same species, the following ajowan essential oil-amoxicillin combinations had additive effect:

- 0.015 mg/ml Ajw – 2 µg/ml Ax (FICI=0.62);
- 0.06 mg/ml Ajw – 2 µg/ml Ax (FICI=0.98);
- 0.06 mg/ml Ajw – 0.25 µg/ml Ax (FICI=0.54) (fig.8.5.c).

The remaining combinations had indifferent effects (tab.8.3.).
Ajowan essential oil/thymol-amoxicillin combinations had indifferent effects against the testes clinical isolates of *Streptococcus pneumoniae*, with the following three exceptions:

- *Streptococcus pneumoniae* 4423:
  - 0.06 mg/ml Ajw – 2 µg/ml Ax – additive effect (FICI=0.98) (fig.8.5.m);
  - 0.03 mg/ml Tim – 2 µg/ml Ax – additive effect aditiv (FICI=1) (fig.8.5.n).

- *Streptococcus pneumoniae* 4409:
  - 0.125 mg/ml Ajw – 0.06 µg/ml Ax – additive effect (FICI=0.98) (tab.8.3.; fig.8.5.e).

Ajowan essential oil/thymol-amoxicillin combinations had indifferent effects against *Pseudomonas aeruginosa* 2351 (tab.8.3.; fig.8.5.o,p).

As to *Moraxella catarrhalis* strains, most ajowan essential oil/thymol-amoxicillin combinations had indifferent effects (tab.8.3.). Exceptions were the following combinations:

- 0.06 mg/ml Tim – 2 µg/ml Ax – additive effect (FICI=0.54) against *Moraxella catarrhalis* 2002 (fig.8.5.r);
- 0.06 mg/ml Tim – 16 µg/ml Ax – additive effect (FICI=0.98) against *Moraxella catarrhalis* 4708 (fig.8.5.t).

This result is important given that most *Moraxella catarrhalis* strains are resistant to amoxicillin, so its association with thymol could restore the effect of amoxicillin on this species.

![Diagram](image1.png)

(a) *Staphylococcus aureus* 37 (clinical isolate), Ajw–Ax combination

![Diagram](image2.png)

(b) *Staphylococcus aureus* 37 (clinical isolate), Tim–Ax combination
(c) *Staphylococcus aureus* 4185 (clinical isolate), Ajw-Ax combination

(d) *Staphylococcus aureus* 4185 (clinical isolate), Tim-Ax combination

(e) *Streptococcus pneumoniae* 4409 (clinical isolate), Ajw-Ax combination

(f) *Streptococcus pneumoniae* 4409 (clinical isolate), Tim-Ax combination

(g) *Streptococcus pneumoniae* 4566 (clinical isolate), Ajw-Ax combination

(h) *Streptococcus pneumoniae* 4566 (clinical isolate), Tim - Ax combination
(i) *Streptococcus pneumoniae* 4546 (clinical isolate), Ajw-Ax combination

(j) *Streptococcus pneumoniae* 4546 (clinical isolate), Tim-Ax combination

(k) *Streptococcus pneumoniae* 4732 (clinical isolate), Ajw-Ax combination

(l) *Streptococcus pneumoniae* 4732 (clinical isolate), Tim - Ax combination

(m) *Streptococcus pneumoniae* 4423 (clinical isolate), Ajw-Ax combination

(n) *Streptococcus pneumoniae* 4423 (clinical isolate), Tim - Ax combination
Fig. 8.5. Isobolograms of *Trachyspermi fructus* essential oil/thymol–amoxicillin combinations
(Ajw= ajowan essential oil, Tim=thymol, Ax=amoxicillin)
8.6. Study of the interactions between *Trachyspermi fructus* essential oil and thymol and ciprofloxacin by checkerboard method

The combination of 2 mg/ml thymol (*Tim*) - 2 mg/ml ciprofloxacin (*Cip*) had an additive effect (FICI = 0.75) against *Staphylococcus aureus* 37 (tab.8.4.; fig.8.6.a).

Ajowan essential oil/thymol-ciprofloxacin (*Ajw/Tim-Cip*) combinations had either additive or indifferent effects against *Staphylococcus aureus* 4185 strain. The following combinations had additive effect:

- 0.03 mg/ml Ajw – 2 µg/ml Cip (FICI=0.74);
- 0.06 mg/ml Ajw – 2 µg/ml Cip (FICI=0.98);
- 0.06 mg/ml Ajw – 1 µg/ml Cip (FICI=0.73);
- 1 mg/ml Tim – 2 µg/ml Cip (FICI=0.62);
- 4 mg/ml Tim – 2 µg/ml Cip (FICI=1);
- 4 mg/ml Tim – 1 µg/ml Cip (FICI=0.75) (tab.8.4.; fig.8.6.c,d).

For the clinical isolates of *Streptococcus pneumoniae*, the effects of ajowan essential oil/thymol-ciprofloxacin combinations ranged from addition to indifference, except for the combination 0.03 mg/ml Tim - 0.5 mg/ml Cip which had synergistic effect (FICI = 0.49) against *Streptococcus pneumoniae* 4423.

The following combinations had additive effects:
- against *Streptococcus pneumoniae* 4566:
  - 0.03 mg/ml Ajw – 0.25 µg/ml Cip (FICI=0.74);
  - 0.06 mg/ml Tim – 0.25 µg/ml Cip (FICI=0.74);
  - 0.125 mg/ml Tim – 0.25 µg/ml Cip (FICI=1);
  - 0.125 mg/ml Tim – 0.125 µg/ml Cip (FICI=0.75) (fig.8.6.g,h);
- against *Streptococcus pneumoniae* 4546:
  - 0.03 mg/ml Ajw – 0.25 µg/ml Cip (FICI=0.74);
  - 0.06 mg/ml Ajw – 0.25 µg/ml Cip (FICI=0.98);
  - 0.125 mg/ml Tim – 0.25 µg/ml Cip (FICI=0.75);
  - 0.25 mg/ml Tim – 0.25 µg/ml Cip (FICI=1);
  - 0.25 mg/ml Tim – 0.125 µg/ml Cip (FICI=0.75) (fig.8.6.i,j);
- against *Streptococcus pneumoniae* 4423:
  - 0.015 mg/ml Ajw – 1 µg/ml Cip (FICI=0.75) (tab.8.4.; fig.8.6.m).

The remaining ajowan essential oil/thymol-ciprofloxacin combinations had indifferent effects.

All ajowan essential oil/thymol-ciprofloxacin combinations had indifferent effects against *Moraxella catarrhalis* strains (1<FICI≤2) (fig.8.6.q,r,s,t).
Indifferent effects were observed for ajowan essential oil/thymol-ciprofloxacin combination against *Pseudomonas aeruginosa* 2351 (1<FICI≤2) (tab.8.4.; fig.8.6.o,p).

(a) *Staphylococcus aureus* 37 (clinical isolate), Ajw-Cip combination

(b) *Staphylococcus aureus* 37 (clinical isolate), Tim-Cip combination

(c) *Staphylococcus aureus* 4185 (clinical isolate), Ajw-Cip combination

(d) *Staphylococcus aureus* 4185 (clinical isolate), Tim-Cip combination

(e) *Streptococcus pneumoniae* 4409 (clinical isolate), Ajw-Cip combination

(f) *Streptococcus pneumoniae* 4409 (clinical isolate), Tim-Cip combination
(g) *Streptococcus pneumoniae* 4566 (clinical isolate), Ajw-Cip combination

(h) *Streptococcus pneumoniae* 4566 (clinical isolate), Tim-Cip combination

(i) *Streptococcus pneumoniae* 4546 (clinical isolate), Ajw-Cip combination

(j) *Streptococcus pneumoniae* 4546 (clinical isolate), Tim-Cip combination

(k) *Streptococcus pneumoniae* 4732 (clinical isolate), Ajw-Cip combination

(l) *Streptococcus pneumoniae* 4732 (clinical isolate), Tim-Cip combination
(m) *Streptococcus pneumoniae* 4423 (clinical isolate), Ajw-Cip combination

(n) *Streptococcus pneumoniae* 4423 (clinical isolate), Tim-Cip combination

(o) *Pseudomonas aeruginosa* 2351 (clinical isolate), Ajw-Cip combination

(p) *Pseudomonas aeruginosa* 2351 (clinical isolate), Tim-Cip combination

(q) *Moraxella catarrhalis* 2002 (clinical isolate), Ajw-Cip combination

(r) *Moraxella catarrhalis* 2002 (clinical isolate), Tim-Cip combination
Ajowan fruits are a customary condiment in Asian food having a thyme-like flavor because they contain thymol. Ajowan essential oil is rich in thymol (35-60%), has a strong antimicrobial activity and, therefore, is of particular interest in therapeutics, both because of its actual effects and its ability to potentiate in combination with various antibiotics their activity.

The conducted study revealed some positive interactions (synergism: $FICI \leq 0.5$ and additivity: $0.5 < FICI \leq 1$) of ajowan essential oil/thymol-amoxicillin combination against clinical isolates of *Staphylococcus aureus*. Analyzing the obtained results, a 4-8 times decrease in amoxicillin MIC with ajowan essential oil/thymol-amoxicillin combination against *Staphylococcus aureus* 37 strain was found. Against the clinical isolate of *Staphylococcus aureus* 4185, amoxicillin MIC decreased 2-16 times with ajowan essential oil-amoxicillin combinations. Thymol-amoxicillin combinations determined a decrease in amoxicillin MIC 4-8 times.

As to the clinical isolate of *Streptococcus pneumoniae* 4409, ajowan essential oil-amoxicillin combination lead to a 2 times decrease in amoxicillin MIC. Against *Streptococcus pneumoniae* 4423, with the combinations ajowan essential oil/thymol-amoxicillin with positive effects, amoxicillin MIC decreased 2 times.

Most *Moraxella catarrhalis* strains are resistant to amoxicillin and amoxicillin association with thymol could restore its effect. Against *Moraxella catarrhalis* 2002 thymol-amoxicillin combination reduced amoxicillin MIC 16 times. Against *Moraxella catarrhalis* 4708, the additive thymol-amoxicillin combination lead to 2 times decreases of amoxicillin MIC.
Ajowan essential oil/thymol-ciprofloxacin combination has a series of additive effects with a 2 times decrease of ciprofloxacin MIC against *Staphylococcus aureus* isolates. Some combinations were proved to have additive effects against clinical isolates of *Streptococcus pneumoniae*. Thus, the combination with ajowan essential oil and thymol lead to a 2-4 times reduction in ciprofloxacin MIC. Combinations with ciprofloxacin were indifferent against clinical isolates of *Pseudomonas aeruginosa* and *Moraxella catarrhalis*.

This study showed positive interactions (synergism, additivity) when ajowan essential oil/thymol were combined with amoxicillin or ciprofloxacin in various ratios. The identification of such combinations with synergistic/additive effects is of importance as it makes possible a reduction of antibiotic dose and implicitly of side effects (gastrointestinal dysfunction, hypersensitivity to amoxicillin, and gastrointestinal, hepatobiliary, sensory, musculoskeletal and connective tissue disorders related to the use of ciprofloxacin) (133).

The synergistic/additive interactions of the combinations in various ratios of ajowan essential oil/thymol with amoxicillin or ciprofloxacin may partly be explained by the ability of thymol to produce structural and functional changes in the cytoplasmic membrane. Thymol mainly affects the lipid membrane structures thereby increasing membrane permeability with massive potassium and ATP losses. In addition, thymol forms a complex with membrane-bound or periplasmic proteins, directly or indirectly affecting the activity of enzymes involved in ATP synthesis and citric acid cycle (226, 227). With the increase in membrane permeability, thymol facilitates the activity and penetration of some substances into the bacterial cell, in this case amoxicillin and ciprofloxacin, fact explaining the synergistic interactions revealed in the present study. The two antibiotics affect the bacterial cell through other mechanisms. Amoxicillin acts primarily at the level of bacterial wall by inhibiting the consolidation process of peptidoglycan in its composition. In contrast, ciprofloxacin blocks cell division by inhibiting DNA gyrase and topoisomerase IV (228).

At the same time, the finding of some indifferent combinations and the absence of antagonistic interactions suggest the possibility of co-administration of preparations containing Ajowan essential oil/thymol together with amoxicillin and ciprofloxacin in infections caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*.

The literature mentions presents examples of synergistic/additive interactions at the association of thymol/plant extracts rich in thymol to various antibiotics and other plant extracts against numerous pathogenic
bacteria, both Gram-positive (Staphylococcus aureus, Bacillus cereus, Listeria innocua) and Gram-negative (Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, Enterobacter cloacae, Pseudomonas fluorescens) (212).

The literature also mentions indifferent or even antagonistic interactions at the association of thymol to other antimicrobial agents. Thus, different combinations of thymol with linalool, carvacrol, borneol, p-cymene, $\alpha$ and $\gamma$-terpineol were indifferent/antagonistic against Staphylococcus aureus ATCC 12600, Enterococcus faecalis ATCC 29212, Bacillus cereus ATCC 11778, Moraxella catarrhalis ATCC 23246, Escherichia coli ATCC 8739 (229). Thymol- penicillin combination was antagonistic against methicillin-resistant Staphylococcus aureus ATCC 25923 (230).
CONCLUSIONS. DEGREE OF ORIGINALITY. RESEARCH PERSPECTIVE

Despite the progress in prophylaxis and treatment in recent years, infectious diseases are still responsible for one third of the deaths worldwide, justifying the interest in the identification of effective antimicrobial agents.

This study evaluated the antimicrobial activity of some extracts/substances of plant origin against bacterial strains, most of them resistant to antibiotics, isolated from patients with lower respiratory tract infections; also evaluated were the interactions that occur when they are combined with conventional antibiotics (amoxicillin, ciprofloxacin).

The conducted studies lead to the following conclusions:

The essential oil of Pimpinella anisum L. fruits (Apiaceae, anise) with a trans-anethole content of 90.18%, acted differently on the tested clinical isolates. The clinical isolates of Moraxella catarrhalis 2002 and 4708 were the most sensitive to the action of this essential oil (MIC=MBC=0.7 mg/ml, and MIC=0.3 mg/ml, MBC=0.7 mg/ml, respectively). Clinical isolates of Gram-positive Streptococcus pneumoniae (4409, 4566, 4546, 4732, 4423) were more resistant to the action of this essential oil (MIC=3.125-25 mg/ml, MBC=6.25-25 mg/ml). The two clinical isolates of methicillin-resistant Staphylococcus aureus (37, 4185) were the least sensitive to the action of anise essential oil (MIC=50 mg/ml, MBC=100 mg/ml and MIC=MBC=100 mg/ml, respectively).

Trans-anethole, the major constituent of anise essential oil acted in a similar manner. Trans-anethole was very active against the clinical isolate of Moraxella catarrhalis 2002 (MIC=0.03 mg/ml, MBC=0.07 mg/ml) and less active against clinical isolates of Moraxella catarrhalis 4708 (MIC=0.3 mg/ml, MBC=0.7 mg/ml) and Streptococcus pneumoniae 4409, 4566, 4546, 4732, 4423 (MIC=0.3-2.5 mg/ml, MBC=0.6-2.5 mg/ml). Methicillin-resistant Staphylococcus aureus strains (32, 4185) were also the least sensitive to the action of trans-anethole (CMI=5 mg/ml, MBC=10 mg/ml). Excepting the clinical isolate of Moraxella catarrhalis 4708, trans-anethole was more active than anise essential oil against all other tested strains.

Of the anise essential oil-amoxicillin combinations we identified a combination with additive effect (FICI=0.98) against Streptococcus pneumoniae 4566, two combinations with additive effect (FICI=0.62, FICI=0.75), one combination with synergistic effect (FICI=0.50) against Streptococcus pneumoniae 4732, and two combinations with antagonistic
effects (FICI=4.71, FICI=4.46) against Moraxella catarrhalis 2002. The anise essential oil-amoxicillin combination decreased amoxicillin MIC 2-4 times against the clinical isolates of Streptococcus pneumoniae 4566 and 4732. Additive effects (FICI=0.59) were found for only one anise essential oil-ciprofloxacin combination against Streptococcus pneumoniae 4566; the association with anise essential oil decreased ciprofloxacin MIC 2 times. The remaining combinations were indifferent (1<FICI≤2).

The anethole-amoxicillin combination with additive effect (FICI=0.75) determined a 4-fold decrease of amoxicillin MIC against the clinical isolate of Moraxella catarrhalis 2002. Anethole-ciprofloxacin combination proved to have additive effects (FICI=0.62, FICI=0.74, FICI=0.73, FICI=0.60) against Streptococcus pneumoniae 4566 strain. For one of them, ciprofloxacin MIC remained constant. For the other combinations there was a 2-8 fold decrease in ciprofloxacin MIC.

In conclusion, the absence of antagonist interactions suggests the possibility of coadministration of preparations containing anise essential oil/anethole and amoxicillin or ciprofloxacin in infections caused by Streptococcus pneumoniae. The identification of antagonist combinations against any of the two clinical isolates of Moraxella catarrhalis suggests caution in the co-administration of anise essential oil/anethole and amoxicillin in infections caused by these bacterial species. To establish the significance of this antagonism, a further study on a larger number of clinical isolates is needed.

The essential oil from Trachyspermum ammi L. (Apiaceae, ajowan) fruits, containing 50.75% thymol, inhibited the growth of clinical isolates of Staphylococcus aureus (37, 4185), Streptococcus pneumoniae (4409, 4566, 4546, 4732, 4423) and Moraxella catarrhalis (2002, 4708) with MIC values ranging from 0.06 to 0.5 mg/ml; against the same strains, the MBC values ranged from 0.06 to 1 mg/ml. Ajowan essential oil was more active than thymol against all tested strains.

Clinical isolates of methicillin-resistant Staphylococcus aureus (37, 4185) were more sensitive to the action of ajowan essential oil (MIC=MBC=0.125 mg/ml) than thymol (MIC=MBC=8 mg/ml).

Of the ajowan essential oil-amoxicillin combinations we identified three combinations with synergistic effect (FICI=0.37, FICI=0.49, FICI=0.36) against Staphylococcus aureus 37 strain. Ajowan essential oil-amoxicillin combination resulted in a 4-8 times decrease of amoxicillin MIC against this strain. Three ajowan essential oil-amoxicillin combinations proved additive effects (FICI=0.62, FICI=0.98, FICI=0.54) against the clinical isolate of Staphylococcus aureus 4185. In these
combinations amoxicillin MIC decreased 2-16 times. One ajowan essential oil-amoxicillin combination with additive effects (FICI=0.98) was identified against *Streptococcus pneumoniae* 4409 and another against *Streptococcus pneumoniae* 4423; in both combinations amoxicillin MIC was 2-fold lower. The remaining ajowan essential oil-amoxicillin combinations had indifferent effects (1 < FICI ≤ 2).

**Ajowan essential oil-ciprofloxacin** combination determined additive effects (FICI=0.74, FICI=0.98, FICI=0.73) against the clinical isolate of *Staphylococcus aureus* 4185; in these combinations ciprofloxacin MIC decreased 2 times. One ajowan essential oil-ciprofloxacin combination with additive effect (FICI=0.74) against *Streptococcus pneumoniae* 4566, two combinations with additive effects (FICI=0.74, FICI=0.98) against *Streptococcus pneumoniae* 4546 and one combination with additive effect against *Streptococcus pneumoniae* 4423 (FICI = 0.73) were identified. In these combinations ciprofloxacin MIC decreased 2-4 times. The remaining ajowan essential oil-ciprofloxacin combinations had indifferent effects (1 < FICI ≤ 2).

**Thymol-amoxicillin** combination had synergistic (FICI=0.5) and additive effects (FICI=0.75, FICI=0.62) against *Staphylococcus aureus* 37 strain. In these combinations amoxicillin MIC was 4-8 times lower. Thymol-amoxicillin combinations had synergistic (FICI=0.37) and additive effects (FICI=0.75, FICI=0.62) against *Staphylococcus aureus* 4185. In these combinations amoxicillin MIC was 4-8 fold lower. Against *Streptococcus pneumoniae* 4423 strain one thymol-amoxicillin combination had additive effect (FICI=1), amoxicillin MIC being 2-fold lower. A thymol-amoxicillin combination with additive effects against *Moraxella catarrhalis* 2002 (FICI=0.54), in which amoxicillin MIC was 16-fold lower was identified. Against the other clinical isolate, *Moraxella catarrhalis* 4708, in thymol-amoxicillin combination with additive effect (FICI=0.98) amoxicillin MIC decreased 2 times.

Against *Staphylococcus aureus* strain 4185** thymol-ciprofloxacin** combination led to additive effects (FICI=0.62, FICI=1, FICI=0.75) in which ciprofloxacin MIC decreased 2-4 times. Of the thymol-ciprofloxacin combinations, three had additive effects (FICI=0.74, FICI=1, FICI=0.75) against *Streptococcus pneumoniae* 4566, three combinations had additive effects (FICI=0.75, FICI=1, FICI=0.75) against *Streptococcus pneumoniae* 4546 and one combination synergistic effect (FICI=0.49) against *Streptococcus pneumoniae* 4423. Ciprofloxacin MIC in these combinations decreased 2 to 4 times. The remaining thymol-ciprofloxacin combinations showed indifferent effects (1 < FICI ≤ 2).
The absence of antagonistic interactions suggests the possibility of combining the ajowan essential oil/thymol preparations with amoxicillin and ciprofloxacin in infections caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*.

The essential oil from *Eugenia caryophyllata* Thunb. (*Myrtaceae*, cloves), having eugenol as the main constituent, (76.51%) was active against all tested clinical isolates. The most sensitive to the action of the essential oil were the clinical isolates of Gram-negative *Moraxella catarrhalis* 2002 (MIC=0.03 mg/ml, MBC=0.07 mg/ml) and 4708 (MIC=0.07 mg/ml, MBC=0.15 mg/ml), followed by Gram-positive *Streptococcus pneumoniae* 4732 (MIC=MBC= 0.3 mg/ml), 4423 and 4566 (MIC=0.3 mg/ml, MBC=1.25 mg/ml), 4546 (MIC=0.6 mg/ml, MBC=0.15 mg/ml) and 4409 (MIC=MBC=0.6 mg/ml). The most resistant to the action of essential oil were the two clinical isolates (37, 4185) of methicillin-resistant *Staphylococcus aureus* (MIC=1.25 mg/ml, MBC=2.5 mg/ml).

Eugenol was less active than the clove essential oil against the clinical isolates of Gram-negative *Moraxella catarrhalis* 2002 (MIC=MBC=0.3 mg/ml) and 4708 (MIC=0.15 mg/ml, MBC=0.3 mg/ml). Against the clinical isolates of *Staphylococcus aureus* (MIC=0.6 mg/ml and 1.25 mg/ml, MBC=2.5 mg/ml), and *Streptococcus pneumoniae* (MIC=0.3 and 0.6 mg/ml, MBC=0.3-1.25 mg/ml) eugenol showed similar or superior effects to clove essential oil.

Clove essential oil -amoxicillin combination had an additive effect (FICI=0.75) against *Streptococcus pneumoniae* 4546; amoxicillin MIC showed a 2-fold decrease in the combination. The remaining clove essential oil-amoxicillin combinations had indifferent effects (1<FICI≤2). All clove essential oil-ciprofloxacin combinations were indifferent.

In the case of eugenol-amoxicillin combinations two combinations with additive effects (FICI=0.96, FICI=0.73) against *Streptococcus pneumoniae* 4546 and one a synergistic combination (FICI=0.35) against *Moraxella catarrhalis* 2002 were identified. The association with eugenol resulted in a 2 and 4 times decrease in amoxicillin MIC against *Streptococcus pneumoniae* 4546 (additive combinations) and 8 times against *Moraxella catarrhalis* 2002 (synergistic combination). Also identified was one eugenol-ciprofloxacin combination with synergistic effects (FICI=0.32) against *Streptococcus pneumoniae* 4546 in which ciprofloxacin MIC decreased 8 times. The remaining eugenol-amoxicillin and eugenol-ciprofloxacin combinations were indifferent (1<FICI≤ 2).
No antagonist interactions were identified in the case of clove essential oil/eugenol-amoxicillin or ciprofloxacin combinations, fact suggesting the safety of co-administration.

The main constituents of the essential oil from *Elletaria cardamomum L. Maton* seeds (*Zingiberaceae*, cardamom) are eucalyptol (1,8-cineole) (31.27%) and α-terpinyl acetate (39.59%). Cardamom essential oil was active against Gram-negative clinical isolates of *Moraxella catarrhalis* 2002 (MIC=MBC=0.15 mg/ml) and 4708 (MIC=MBC=0.07 mg/ml); its effects on clinical isolates of *Streptococcus pneumoniae* (4409, 4566, 4546, 4732, 4423) (MIC=0.12-1.5 mg/ml, MBC=0.15-3.125 mg/ml) and *Staphylococcus aureus* 37 and 4185 (MIC=0.26 mg/ml, MBC=12.5 mg/ml) were much lower. The high resistance of the two strains of methicillin-resistant *Staphylococcus aureus* to the action of essential oil was evident. Eucalyptol was more active than cardamom essential oil against most of the tested strains.

**Cardamom essential oil-amoxicillin** combination had an additive effect (FICI=0.53) against *Escherichia coli* ATCC 25922; amoxicillin MIC decreased 16 times.

Cardamom essential oil-amoxicillin combinations had additive effects (FICI=0.61, FICI=1, FICI=0.56, and FICI = 0.51, respectively) against the two clinical isolates of *Staphylococcus aureus*. In these combinations amoxicillin MIC decreased 2-16 times. Against *Moraxella catarrhalis* 2002 strain cardamom essential oil-amoxicillin combination had an additive effect (FICI=0.71), amoxicillin MIC decreasing 4 times. **Cardamom essential oil-ciprofloxacin** combinations showed additive effects (FICI=0.53, FICI=1) against the reference strain *Staphylococcus aureus* ATCC 25923 and clinical isolate of *Staphylococcus aureus* 4185. In these combinations ciprofloxacin MIC decreased 4 and 2 times, respectively.

**Eucalyptol-amoxicillin** combination had additive effects against *Staphylococcus aureus* 4185 (FICI=0.74), amoxicillin MIC decreasing 2 times. Additive effects were also identified (FICI=1, FICI=0.98) against *Streptococcus pneumoniae* 4732 and 4423 in which amoxicillin MIC decreased 2 times. For the clinical isolate of *Moraxella catarrhalis* 2002 eucalyptol-amoxicillin combination determined an additive effect (FICI=0.64) decreasing amoxicillin MIC 4 times. All eucalyptol-ciprofloxacin combinations determined indifferent effects (1<FICI≤2).

Cardamom essential oil and eucalyptol did not antagonize the effects of amoxicillin and ciprofloxacin against clinical isolates of *Staphylococcus aureus, Streptococcus pneumoniae* and *Moraxella catarrhalis*, suggesting
the possibility of co-administration without a reduction in the effectiveness of the antibiotic.

Extracts from the aerial parts of *Achillea distans* Waldst. & Kit. ex Willd. (A1) and *Achillea setacea* Waldst. & Kit. ex Willd. (A2) (*Asteraceae, tall yarrow*), with a total polyphenolic content of 9.21±0.25% and 7.71±0.25%, respectively, were more active against the clinical isolates of *Moraxella catarrhalis* 2002 and 4708 (MIC=0.15 mg/ml, MBC=0.6 mg/ml for the A1; MIC=0.15 mg/ml, MBC=0.3 mg/ml for A2). The antibacterial effects of the two extracts against the clinical isolates of *Streptococcus pneumoniae* were lower (MIC=1.25 mg/ml, MBC=5 and 10 mg/ml).

A1/A2-amoxicillin combinations determined additive effects (FICI=0.52, FICI=0.71, FICI=0.60, FICI=0.54, FICI=0.52, FICI=0.52) against *Streptococcus pneumoniae* 4566, 4646, 4732, 4423, and *Moraxella catarrhalis* 2002 and 4708. In these combinations amoxicillin MIC decreased 4, 8 and 16 times.

The remaining A1/A2-amoxicillin combinations were indifferent (1<FICI≤2).

A1/A2-ciprofloxacin combinations caused a synergistic effect (FICI=0.49) against *Streptococcus pneumoniae* 4409, and additive effects (FICI=0.73, FICI=0.96) against *Streptococcus pneumoniae* 4566, 4546, 4732, 4423, and *Moraxella catarrhalis* 2002 and 4708. In all these combinations ciprofloxacin MIC decreased 2-4 times.

A1 and A2 extracts did not antagonize the effects of amoxicillin and ciprofloxacin against *Streptococcus pneumoniae* and *Moraxella catarrhalis* isolates, suggesting the possibility of co-administration in the case of infections caused by the above mentioned bacterial species.

In the methanolic extract (H2) from inflorescences of *Helichrysum arenarium* L. Moench (*Asteraceae, everlasting*) with a total polyphenol content of 16.01±0.09%, the following compounds were identified by HPLC-DAD-ESI-MS: quinic, chlorogenic and caffeic acids, two dicaffeoylquinic acids, three naringerin-0-hexosides, apigeninl-7-O-glucoside, together with naringerin, apigenol and kaempferol. Against the clinical isolate of *Moraxella catarrhalis* 8123 the extract showed a good antibacterial activity (MIC=0.15 mg/ml and MBC=5 mg/ml). According to MIC values (1.25 and 2.5 mg/ml), the antibacterial effects of H2 extract against clinical isolates of *Staphylococcus aureus* (37 and 4185) and *Streptococcus pneumoniae* (7129, 5693, 7149, 7150, 5691) were more reduced.
Both H2-ciprofloxacin combinations showed additive effects (FICI=0.62) against standard strains and the two clinical isolates of *Staphylococcus aureus*, ciprofloxacin MIC decreasing 8 times.

Against the clinical isolates of pneumococci (7149, 7150, 5693, 5691) H2-ciprofloxacin combinations had synergistic (FICI=0.49) and additive effects (FICI=0.75). In these combinations ciprofloxacin MIC decreased 4 times.

This study revealed positive interactions (synergism, additivity) when plant essential oils/extracts were combined in various ratios with amoxicillin or ciprofloxacin. The identification of such combinations with synergistic/additive effects is important allowing to reduce the antibiotic dose and thus its side effects.

Although the antimicrobial effects of plant extracts/substances have been extensively studied, the interactions that occur when combining them with antibiotics are less known. Plant extracts are multicomponent mixtures in which the constituents act through different mechanisms, which explains the interaction complexity when co-administered with other substances. The conducted study showed that the plant extract/substance-antibiotic ratio significantly influences the nature of the interaction.

The nature of interactions is not only influenced by the plant-drug ratio, but also by the chemical composition of the extract, both qualitative and quantitative, metabolic, physiological and pathogenicity characteristics of microorganisms.

It is obvious that for an effective use of extracts/substances of plant origin knowing the exact plant-antibiotic ratios at which positive interactions do occur, and the mechanism of antimicrobial action at cell, molecular, and genetic level are essential.

- **Degree of originality. Research perspectives**

My original contribution to present knowledge is:

- assessment of the antibacterial effects of some essential oils *Aetheroleum Anisi, Aetheroleum Trachyspermi, Aetheroleum Caryophylli, Aetheroleum Cardamomi* and their major constituents (anethole, thymol, eugenol, eucalyptol) against clinical isolates (*Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis*); study of the interactions that may occur when combined with amoxicillin and ciprofloxacin;
- assessment of the antibacterial effects of extracts from flowering aerial parts of *Achillea distans* and *Achillea setacea* against standard strains
(Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and clinical isolates (Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis), as well as of the interactions occurring in combination with amoxicillin and ciprofloxacin;

- study of the polyphenolic spectrum in the inflorescences of Helichrysum arenarium subsp. arenarium by RP-HPLC-ESI-MS-DAD; study of the antibacterial effects of the methanolic extract from inflorescences against standard strains (Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853) and the clinical isolates (Staphylococcus aureus, Streptococcus pneumoniae, Moraxella catarrhalis) and of the interactions occurring in combination with ciprofloxacin;

- screening of the antibacterial effects of dichloromethane, methanol and aqueous extracts from the flowering aerial parts of Scutellaria altissima L. against standard strains (Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853).

**Research perspectives**

The results warrant further studies in the following areas:

- assessment of the effects of plant extracts/substances-antibiotic combinations which have synergistic, additive and antagonistic effects on a larger number of clinical isolates;
- elucidation of the mechanisms of action of plant extracts/substances;
- *in vivo* assessments of the interactions generated by these combinations;
- further study on other standard bacterial strains and clinical isolates.
SELECTIVE REFERENCES


23. Bowker CE, Garvey MI, Noel AR, Tomaselli SG, MacGowan AP. Comparative antibacterial effects of moxifloxacin and levofloxacin on *Streptococcus pneumoniae* strain with defined mechanisms of


ANNEX 1. Published papers on dissertation topic

Articles published in ISI journals


Articles published in B+ (BDI) journals


Papers presented in national conferences