Pharmaceutical Forms with Basil and Propolis 
to the Benefit of the Oral Cavity 
Formulation, preparation and microbiological analysis

OLIMPIA DUMITRIU BUZIA*, NELA MARDAREⁱ, RAMONA DRAGOMIRⁱ, MAGDALENA MIULESCU⁵, ALIN LAURENTIU TATU³

¹ Dunarea de Jos University, Faculty of Medicine and Pharmacy, Research Center in the Field of Medical and Pharmaceutical Sciences, 35 Al. I. Cuza Str., 800010, Galati, Romania
² College of Pharmacists, 27 Galati Str., 810388, Galati, Romania
³ Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, Department of Morphological and Functional Sciences, 35 Al. I. Cuza Str., 800010, Galati, Romania

The present paper aims at formulating, preparing, following the antibacterial activity of pharmaceutical forms with oral cavity applicability. Two pharmaceutical forms were prepared, a tincture and a toothpaste based on basil and propolis, being subsequently compared to the mix of basil and propolis tinctures that are commercially available; each pharmaceutical form was analyzed organoleptically and microbiologically. As a result of the measurements the conclusion was drawn that the tinctures prepared, the propolis tincture and the basil tincture show a strong sensitivity Staphylococcus aureus, Pseudomonas aeruginosa; and Enterococcus hormaechei. Candida albicans reacted more strongly to the propolis tincture, and less to the basil tincture. The scientific novelty of the paper consists in the formulation and determination of the antimicrobial activity of the toothpaste prepared in the laboratory of the Pharmacy Faculty of Galati. The results obtained were favorable on microorganisms acting on the oral cavity, i.e. gram positive germs, gram negative germs and fungi.

Keywords: basil (Ocimum basilicum) propolis, lemon basil, tincture

In order to heal lesions and various afflictions, man has used plants from times immemorial. Among the studied species are Ocimum basilicum (basil), and also propolis. The essential basil oils (such as ecogole, inalool, cineol, eugenol, sabinen, myrcene and limonen) just like propolis, have shown a strong antibacterial activity [1-3] against germs like Staphylococcus aureus and Escherichia coli and fungi like Candida albicans, or Gram positive bacteria. Propolis has a special pharmacological value, as it is an active natural source for a series of antimicrobials [4].

Man became aware of the need to clean his mouth and teeth at the same time with his desire to maintain his health. Since the Antiquity humans have been confronted with problems at the level of the oral cavity, thus trying to find ways of preserving oral hygiene. The powder of dry basil leaves may be used to brush teeth, due to its beneficial effects in maintaining teeth health and countering bad breath. [International Journal of Pharmaceutical Sciences Review and Research, 2014]

Nowadays toothpastes [5,6] are pharmaceutical forms widely used in the therapy of the oral cavity.

Experimental part

Materials and methods

Natural propolis was used, originating from a bee keeper in the county of Galati, as well as holy basil and lemon basil, originating from the botanical gardens of the Faculty of Pharmacy in Galati. The devices used were the extractor of the faculty in order to obtain the extractive solutions of basil and propolis, distilled water, ethyl alcohol 96°, mortars, pestles, Berzelius beakers, analytical scales, pharmaceutical scales, 10 mL brown flasks. In order to perform microbiological measurements, the following culture media were used: Mueller Hinton, blood-gellosis, solid Sabouraud Fluconazole 25 µg, Levofloxacin 5µg, Ciprofloxacin 25 µg.

The methods used in obtaining tinctures were the methods of alcoholic extractive solutions, according to RFX, and the maceration extraction method, respectively. The propolis tincture obtained had 30% concentration, and the holy and lemon basil tinctures had 20% concentration. The sensitivity/resistance tests for the alcoholic extracts of propolis and holy and lemon basil were performed in the microbiology laboratory of the Faculty of Medicine and Pharmacy within the Sf. Ioan Clinical Emergency Hospital of Galati, through the difussimetric method (Kirby-Bauer).

Results and discussions

Three tinctures were prepared by means of the alcoholic solvent extraction method, and then compared to two commercially available tinctures, as follows:

- Tincture 1- holy basil sown in the Rasvan Angheluta Botanical Gardens in Galai, the seeds originating in Satu Mare;
- Tincture 2- lemon basil sown in the Rasvan Angheluta Botanical Gardens of Galai, the seeds originating in Satu Mare;
- Tincture 3- crude propolis from the Galati area, from a local beekeeper;
- Tinctures 4- commercially available Ocimum extract;
- Tincture 6- commercially available propolis extract;
- Tincture 7- the mixture of tinctures of propolis, holy basil and lemon basil.

Several physical characteristics of tinctures, i.e. appearance, color, odor, taste were studied, the results being shown in table 1.

The toothpaste was manufactured in three variants, according to the guar gum concentration: 1, 2, and 3% according to table 2.

* email: buzia_olimpia@yahoo.com
All authors have equal contribution to designing and writing the presented paper.
The sodium bicarbonate is triturated in a mortar. 10 mL of water is added and stirred until the bicarbonate is dissolved. Separately, 4 g of water are boiled on the stove, to which the lauryl sulphate is added. After cooling it is added to the solution of bicarbonate. The guar gum is added to this solution, triturating until homogenization. In another mortar, 2 g of calcium carbonate are added and triturated with the glycerin until homogenization. The solution prepared previously is mixed with carbonate powder and glycerin, stirring energetically until homogenization. The tinctures are added one by one and at the end the preservative solution and the mint oil are added, triturating until complete homogenization.

Description: light brown paste due to the tinctures, homogeneous, with specific taste and smell.

It may be concluded that out of the preparations the guar gum 2% is the most suitable in point of appearance and properties, similar to what is commercially available.

In view of determining the antimicrobial activity through the Kirby Bauer method, the following bacterial strains and culture medium were used:

- gram negative bacilli: Escherichia coli, Enterobacter hormaechei, Pseudomonas aeruginosa;
- gram positive cocci: Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus; solid Sabouraud for fungi.

We used fluconazole discs 25 µg, Levofloxacin 5µg, Ciprofloxacin known concentrations, and in the culture media performed 6 mm holes where we introduced 50 µL propolis tincture, holy basil tincture, lemon basil tincture, and ethyl alcohol 70°.

For each bacterial strain it was necessary to prepare an inoculum, so that for each bacterial strain isolated in pure culture the inoculum had a turbidity of 0.5 Mc Farland (1.5 x 10^8 CFU / mL), nephelometrically measured by means of the Densimat device.

In about 3 mL saline solution for bacteria or liquid Sabouraud medium for fungi, 5 identical colonies were suspended (fig. 1).

A marker pen was used to put down the data necessary to identify the bacteria to be cultured. A plastic pipette with a severed tip was used to create holes in the culture medium. On the surface of the medium, the culture was performed by means of a sterile cotton swab dipped in the microbial suspension, rinsed by twisting it against the inner wall of the suspension tube in order to remove the excess liquid.

The content of the swab was uniformly discharged on the surface of a solid medium, cultured with the bacterial/fungal suspension to be tested, the active antimicrobial substance spreads in the medium and creates around the disc an area of culture inhibition, according to the sensitivity of the bacterial or fungal culture.

The cultured germ cannot grow in the area where the antibiotic concentration is above the minimal inhibiting concentration.

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Gum 1%</th>
<th>Gum 2%</th>
<th>Gum 3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>1g</td>
<td>1g</td>
<td>1g</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>2g</td>
<td>2g</td>
<td>2g</td>
</tr>
<tr>
<td>Guar gum</td>
<td>0.2g</td>
<td>0.4g</td>
<td>0.6g</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>0.02g</td>
<td>0.02g</td>
<td>0.02g</td>
</tr>
<tr>
<td>Glycerin</td>
<td>1g</td>
<td>1g</td>
<td>1g</td>
</tr>
<tr>
<td>Mint oil</td>
<td>2-3 drops</td>
<td>2-3 drops</td>
<td>2-3 drops</td>
</tr>
<tr>
<td>Propolis tincture</td>
<td>0.4g</td>
<td>0.4g</td>
<td>0.4g</td>
</tr>
<tr>
<td>Holy basil tincture</td>
<td>0.4g</td>
<td>0.4g</td>
<td>0.4g</td>
</tr>
<tr>
<td>Lemon basil tincture</td>
<td>0.4g</td>
<td>0.4g</td>
<td>0.4g</td>
</tr>
<tr>
<td>Preservative solution</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>14g</td>
<td>13.35g</td>
<td>13.35g</td>
</tr>
</tbody>
</table>

The culture media used were the following:

Mueller Hinton for: Enterobacter, Staphylococcus aureus, Escherichia coli; blood gellosis for: Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus; solid Sabouraud for fungi.

We used fluconazole discs 25 µg, Levofloxacin 5µg, Ciprofloxacin known concentrations, and in the culture media performed 6 mm holes where we introduced 50 µL propolis tincture, holy basil tincture, lemon basil tincture, and ethyl alcohol 70°.

In about 3 mL saline solution for bacteria or liquid Sabouraud medium for fungi, 5 identical colonies were suspended (fig. 1).

Table 2

<table>
<thead>
<tr>
<th>FORMULATION OF TOOTHPASTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical properties</td>
</tr>
<tr>
<td>Appearance</td>
</tr>
<tr>
<td>Color</td>
</tr>
<tr>
<td>Odor</td>
</tr>
<tr>
<td>Taste</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Tincture 1</th>
<th>Tincture 2</th>
<th>Tincture 3</th>
<th>Tincture 4</th>
<th>Tincture 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Color</td>
<td>Greenish-black</td>
<td>Greenish-black</td>
<td>Reddish brown</td>
<td>Greenish-black</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Odor</td>
<td>Specific aroma</td>
<td>Specific aroma</td>
<td>Specific aroma</td>
<td>Specific aroma</td>
<td>Specific aroma</td>
</tr>
<tr>
<td>Taste</td>
<td>Slightly burning</td>
<td>Slightly burning</td>
<td>Slightly burning</td>
<td>Slightly burning</td>
<td>Slightly burning</td>
</tr>
</tbody>
</table>

Table 2
When performing holes a considerable distance was kept among them and to the dish edge. The hole-forming pipette diameter was 6 mm.

After applying the minipills and pipetting the extracts in the holes, we allowed medium-adhesion for 10-15 minutes, and then incubation in the thermostat at a temperature of 37°C. The dishes were placed in reversed position, no more than 3 dishes one above the other in aerobiosis conditions, and left to incubate for 24 h.

The antimicrobial substance in the disc spreads in the medium and achieves around the disc an area of culture inhibition, according to its sensitivity degree.

In case the concentration of antibiotic / antimicotic/tincture/ethyl alcohol 70° is above the minimal inhibiting concentration, the cultured germ does not grow in the concentration area.

Upon dish incubation, special attention was granted to reading the dishes that showed culture increase from the point of view of purity and density (almost confluent dense culture).

The reading was performed by means of a graded ruler and the naked eye, measuring the inhibition area diameter in mm, 2 or 3 times, in various directions.

The inhibition area diameters were taken into consideration: the area without microbial colonies visible to the naked eye, including the diameter of the antibiotic disc, the well-developed colonies within the inhibition area.

The overinfected media were disposed of in the special compartment and the test was repeated.

**Reading and expressing results**

If around the hole/ micropill there was growth inhibition, then the result is sensitivity (S).

If the inhibition area diameter was 6 mm, it shows resistance.

For the antibiotics used in testing, the interpretation was performed according to the reference standard CLSI 2014.

The testing of bacterial sensitivity to the antibiotic yielded the results seen in table 3.

If the strain is sensitive, the antibiotic/antimicotic tested may be used in the therapy of the infection caused by the germ in question.

If the strain shows intermediary sensitivity, the antibiotic/antimicotic may be used in treating the infection caused by that particular germ in larger doses than therapeutic doses.

If the strain is resistant, the antibiotic/antimicotic cannot be used in treating the infection caused by the germ in question.

**Results on assessing antimicrobial activity**

The assessment of the antibacterial activity of propolis by the diffusimetric method aimed at measuring the inhibition diameter for the analyzed tinctures and toothpastes. The appearance of the plates after inoculation and incubation may be seen in figures 4-7.

![Fig. 4. Antimicrobial activity on the strain of Enterococcus casseliflavus](image)

![Fig. 5. Antimicrobial activity on the strains of Staphylococcus aureus](image)

![Fig. 6. Antimicrobial activity on the yeast Candida albicans](image)

![Fig. 7. Antimicrobial activity on Streptococcus pyogenes](image)

**Results on assessing the antimicrobial activity of tinctures**

The results obtained after measuring the inhibition diameter for Gram negative bacteria are shown in table 4 and figure 8.

The prepared propolis tincture (T3) manifested a better inhibition capacity on Enterococcus hormaechei and *Pseudomonas aeruginosa* than the commercially available propolis tincture (T6), but the latter had better inhibition capacity on *Escherichia coli*. As compared to the antibiotic tested, the prepared tincture (T3) had lower values. Similar results were obtained by researcher Probs and his team in 2011.

T1 (the holy basil tincture we prepared) showed a lower inhibition capacity on the bacteria *Escherichia coli* and Enterococcus hormaecheias compared to T2 (lemon basil tincture), and the latter had lower inhibition capacity on *Pseudomonas aeruginosa* than T1. The commercially available basil tincture (T4) had better inhibition capacity on Enterococcus hormaechei than T1 and T2, but lower on the other two Gram negative species. The two prepared tinctures, T1 and T2, had almost similar values.

The equal-part mixture of commercially available propolis tincture and commercially available basil tincture, T6, had inhibition capacity on *Enterococcus hormaechei*, while the mixture of prepared tinctures, T7, showed no antimicrobial action (zero activity). As far as the bacteria *Escherichia coli* concerned, T6 worked better than...
Table 4

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>Alcohol 70°</th>
<th>Ciprofloxacin 25µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter hormaechai</td>
<td>12</td>
<td>20</td>
<td>15</td>
<td>18</td>
<td>5</td>
<td>12</td>
<td>-</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>13</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12</td>
<td>10</td>
<td>14</td>
<td>10</td>
<td>12</td>
<td>20</td>
<td>22</td>
<td>8</td>
<td>27</td>
</tr>
</tbody>
</table>

Fig. 9. Antimicrobial action of tinctures, 70° alcohol and antibiotic on Gram positive bacteria

The propolis tincture we prepared (T3) had a better inhibition action on Gram positive bacteria, as compared to tinctures T1 and T2, and better than commercially available tinctures T4 and T5.

The commercially available mixture of tinctures T6 had superior inhibition capacity for *Staphylococcus aureus* and equal for the species *Streptococcus pyogenes*. The strain *Streptococcus pneumoniae* was acted upon only by the mixture of prepared tinctures, T7.

Table 6 and figure 10 show the data on the antifungal activity of tinctures and fluconazole on the strain *Candida albicans*.

Table 5

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>Alcohol 70°</th>
<th>Ciprofloxacin 25µg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>7</td>
<td>8</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td><em>Enterococcus casseliflavus</em></td>
<td>8</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>-</td>
<td>13</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>6</td>
</tr>
</tbody>
</table>

It was noted that the commercially available tincture (T5) had a better inhibition capacity, closer to the antibiotic’s, as compared to the prepared propolis tincture (T3) which showed lower capacity. Basil tinctures displayed similar capacities, the weaker being T2, i.e. lemon basil tincture. The mixtures had the same inhibition capacity.

Upon the analysis of figure 11 it may be seen that the prepared propolis tincture (T3) showed a stronger sensitivisation action Gram positive bacteria.

Table 6

<table>
<thead>
<tr>
<th>Fung</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>Alcohol 70°</th>
<th>T9</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>15</td>
<td>8</td>
<td>17</td>
<td>14</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>6</td>
<td>28</td>
</tr>
</tbody>
</table>

Results on antimicrobial activity of toothpastes:

Toothpastes were named as follows:
- Toothpaste 1: toothpaste with 1% guar gum concentration
- Toothpaste 2: toothpaste with 2% guar gum concentration
- Toothpaste 3: toothpaste formulated without tinctures

Upon performing the tests, good results were obtained with Toothpaste 2 on Gram positive strains, especially *Staphylococcus aureus*. *Enterococcus casseliflavus* was not affected by toothpastes. As compared to the antibiotic used, Toothpaste 2 showed a good activity. Toothpaste 3, without tincture addition, was not effective
Table 7
ANTIMICROBIAL ACTIVITY OF TOOTHPASTES WITH AND WITHOUT TINCTURES ON GRAM POSITIVE BACTERIA

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Toothpaste 1</th>
<th>Toothpaste 2</th>
<th>Toothpaste 3</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>14</td>
<td>20</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>14</td>
<td>15</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>16</td>
<td>18</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Enterococcus cassiflafus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
</tbody>
</table>

Fig.12 Antimicrobial activity of toothpastes and antibiotic on Gram positive bacteria

Table 8
ANTIMICROBIAL ACTION OF TOOTHPASTES AND ANTIBIOTIC ON GRAM NEGATIVE BACTERIA

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Toothpaste 1</th>
<th>Toothpaste 2</th>
<th>Toothpaste 3</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td>17</td>
<td>20</td>
<td>13</td>
<td>38</td>
</tr>
</tbody>
</table>

Fig.13. Action of toothpastes and antibiotic on Gram negative bacteria

Table 9
ANTIMICROBIAL ACTION OF TOOTHPASTES AND ANTIBIOTIC ON FUNGI

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Toothpaste 1</th>
<th>Toothpaste 2</th>
<th>Toothpaste 3</th>
<th>Fluconazole 5ug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>23</td>
<td>24</td>
<td>18</td>
<td>28</td>
</tr>
</tbody>
</table>

Fig.14. Antimicrobial action of toothpastes on the yeast Candida albicans

Conclusions
Three tinctures were prepared, propolis tincture 30%, holy basil and lemon basil tinctures, both in concentration of 20%, whose physical characteristics and antimicrobial capacities were traced, comparing the results with those for commercially available products.

Four toothpastes were prepared, out of which three based on tinctures and various concentrations of guar gum, i.e., 1%, 2% and 3%.

The antimicrobial activity of propolis and basil were assessed in accordance with eight reference strains, Gram positive, Gram negative and the fungus Candida albicans.

It was shown that the propolis tincture proved to be better than the commercially available tincture, and better than the basil tincture. It showed its efficacy on Pseudomonas aeruginosa, Candida albicans and Enterococcus hormaechei.
The basil tinctures had lower antimicrobial efficacy than the propolis tincture, and out of the two species, lemon basil was more efficient in point of inhibition capacity on the fungus *Candida albicans* and *Escherichia coli* (20 mm), as compared to the antibiotic tested (20 mm).

Toothpaste 2 had better antimicrobial activity, which is why in the near future we might create an encapsulated form with retard action with a much more efficient action [7-9]. Our recommendation is to associate it with the extractive solution of Kombucha, taking into account the similar action on microorganisms [10]. It is common knowledge that hospitals are threatened by nosocomial infections due to these killer microorganisms [11]. The adverse effects of different synthetic drugs are wellknown when they are prescribed for different diseases or comorbidities [12-29]. The usage of natural extracts or plant derivates is desirable for their efficacy without major adverse reactions even on normal microbiota, or if needed after the informed consent even in pregnancy [30-42]. It is possible to use these natural products under various pharmaceutical forms, some of them even retard forms, in view of reducing nosocomial infections in hospitals.

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