Pharmaceutical Forms with Basil and Propolis to the Benefit of the Oral Cavity

Formulation, preparation and microbiological analysis

OLIMPIA DUMITRIU BUZIA[1]*, NELA MARDARE[2], RAMONA DRAGOMIR[2], MAGDALENA MIULESCU[3], ALIN LAURENTIU TATU[1]

[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.

Keyworlds: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 positivebacteria*. 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 healthand 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 diffusimetric 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 ofGalai, the seeds originating in Satu Mare;

Tincture 3- brute propolis from the Galati area, from a local beekeeper;

Tincture 4 - commercially available *Ocimum* extract;

Tincture 5- commercially available propolis extract;

Tincture 6- commercially available mixture of propolis and basil tinctures;

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.

Table 1

ORGANOLEPTIC CHARACTERISTICS OF PREPARED TINCTURES VS. COMMERCIALLY AVAILABLE TINCTURES

Physical properties	Tincture 1	Tincture 2	Tincture 3	Tincture 4	Tincture 5
Appearance	Clear	Clear	Clear	Clear	Clear
Color	Greenish-black	Greenish-black	Reddish brown	Greenish-black	Reddish-brown
Odor	Specific aroma	Specific aroma	Specific aroma	Specific aroma	Specific aroma
Taste	Slightly burning	Slight anesthetic sensation	Slightly burning	Slightly burning	Slight anesthetic sensation

Table 2

FORMULATION OF TOOTHPASTE

Components	Gum 1%	Gum 2%	Gum 3%
Sodium bicarbonate	1g	1g	1g
Calcium carbonate	2g	2g	2g
Guar gum	0.2g	0.4g	0.6g
Sodium lauryl sulphate	0.02g	0.02g	0.02g
Glycerin	1g	1g	1g
Mint oil	2-3 drops	2-3 drops	2-3 drops
Propolis tincture	0.4g	0.4g	0.4g
Holy basil tincture	0.4g	0.4g	0.4g
Lemon basil tincture	0.4g	0.4g	0.4g
Preservative solution	1 ml	1ml	1ml
Distilled water	14g	13.38g	13.18g

Preparation technique

The sodium bicarbonate is triturated in a morta10 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 the 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 aeruginoasa*;

-gram positive cocci: *Staphylococcus aureus, Streptococcus pneumoniae*; *Streptococcus pyogenes, Enterococcus casseliflavus*;

-Fungi: *Candida albicans*

-Culture media : Mueller Hinton, blood gellosis , solid Sabouraud

The diffusimetric (Kirby-Bauer) method

Each of the isolated strains were tested for sensitivity to alcoholic extracts obtained from raw propolis, holy basil, and lemon basil, ethyl alcohol 70 degrees and antibiotics (Levofloxacin, Ciprofloxacin) for bacteria, and an antimicotic agent (fluconazole) for fungi.

To prove the antimicrobial activity, the diffusimetric (Kirby-Bauer) antibiogram was used.

By depositing antibiotic micropills 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.

The culture media used were the following:

Mueller Hinton for: *Enterobacter, Staphylococcusaureus, Escherichia coli;*

Blood-gellosis for*: Streptococcus pyogenes, Streptococcus pneumoniae, Enterococcus;*

solid Sabouraud for*fungi.*

We used fluconazole discs 25 µg, Levofloxacin 5µg, Ciprofloxacinin known concentrations, and in the culture mediawe 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).

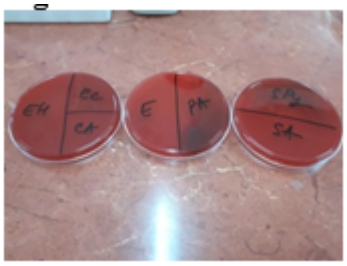

Fig.1. Petri dishes containing the bacterial colonies

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 medium surface by light horizontal movements, without scratching the medium. The inoculum was left to be absorbed in the medium for 5-10 min, then the alcoholic extract was pipetted in each hole, and in the space between holes the discs (minipills with antibiotics, antifungal agents) were applied by means of tweezers, gently pressing on the surface of the cultured medium (fig. 2, 3).

Fig.2 Bacterial inoculum for Enterobacter hormaechei

Fig.3 Bacterial inoculum for Escherichia coli

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 placedin 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 pointof 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.

Table 3

SENSITIVITY OF THE MICROORGANISMS TESTED TOLEVOFLOXACIN, CIPROFLOXACIN, FLUCONAZOLE

		Diameter of the inhibition area	
Antibiotic-Antimicotic	Resistant (R)	Intermediary(I)	Sensitive(S)
Levofloxacin 5µg	≤13 mm	14-16 mm	≥17 mm
Ciprofloxacin 25µg	≤12 mm	13-14 mm	≥15 mm
Fluconazole 25µg	≤14 mm	15-18 mm	≥19 mm

Source: *CLSI Standards 2014*

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 therapeutical 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.

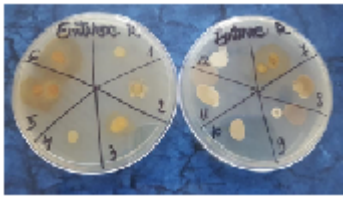

Fig. 4.Antimicrobial activity on the strain of *Enterococcus* casseliflavus

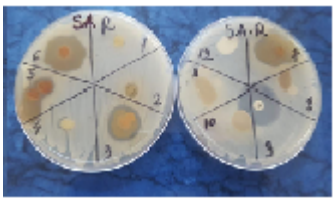

Fig.5.Antimicrobial activity on the strains of *Staphylococcus aureus*

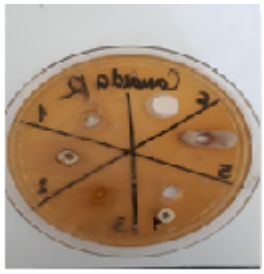

Fig.6. Antimicrobial activity on the yeast *Candida albicans*

Fig. 7. Antimicrobial activity on *Streptococcus pyogenes*

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*Echerichia 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*Enterococcushormaechei* 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*Escherichiacoli*is concerned,T6 worked better than

Microbial strains	T 1	T2	T3	T4	T5	T6	T7	Alcohol 70°	Ciprofloxacin 25µg
Enterobacter hormaechei	12	20	15	18	6	12	-	8	38
Escherichia coli	13	14	15	10	15	20	10	0	32
Pseudomonas aeruginosa	12	10	14	10	12	20	22	6	27

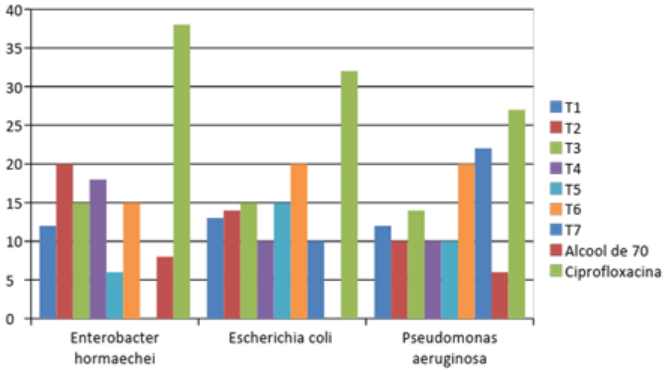

Fig. 8. Diameters of inhibition areas for some Gram negative bacteria

T7, but regarding *Pseudomonas aeruginosa*, T7 had better inhibition capacity.

The results obtained in measuring the inhibition area for Gram positive bacteria are shown in table 5 and figure 9.

Table 5

ANTIBACTERIAL ACTIVITY OF TINCTURES, ALCOHOL, LEVOFLOXACIN ON GRAM POSITIVEBACTERIA

Microbial strains	T1	T2	T3	T4	T5	T6	T7	T8	T9
Staphylococcus aureus	8	10	18	7	8	20	10	0	27
Enterococcus casseliflavus	8	10	-	10	8	10	12	6	24
Streptococcus pyogenes	10	12	13	11	15	20	20	7	20
Streptococcus pneumoniae	-	15	18	-	-	-	15	0	30

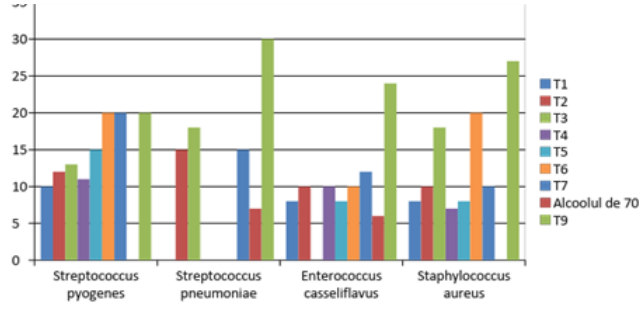

Fig. 9. Antimicrobial action of tinctures, 70° alcohol and antibiotic on Gram positive bacteria

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 4

ANTIMICROBIAL ACTION OF TINCTURES ON GRAM NEGATIVE BACTERIA

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

ANTIFUNGAL ACTIVITY OF TINCTURES, 70° ALCOHOL, AND FLUCONAZOLE

Fungi	T1	T2	T3	T4	T5	T6	T7	Alcohol 70°	T9
Candida albicans	13	8	17	14	10	15	26	6	28

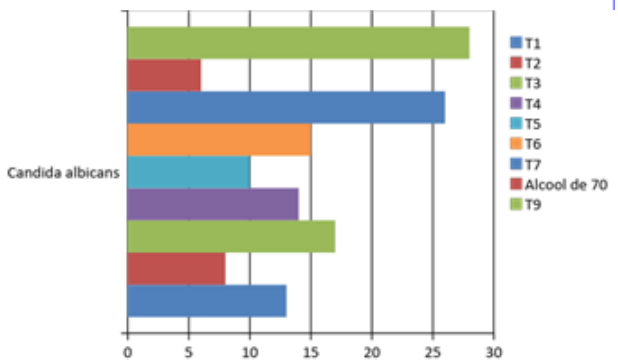

Fig.10. Diameters of inhibition areas for tinctures, alcohol and fluconazole

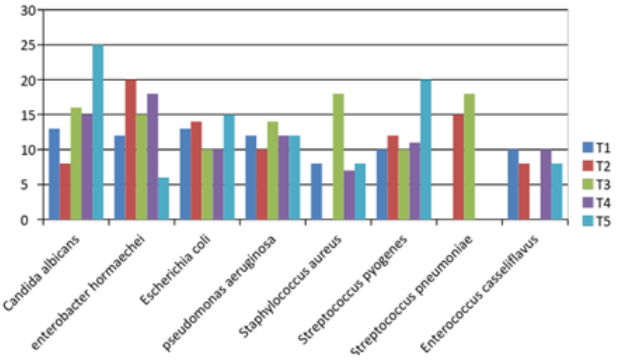

Fig.11. Comparison of antimicrobial activity shown by various tinctures

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 casseliffavus* 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

Bacterial strains	Toothpaste 1	Toothpaste 2	Toothpaste 3	Antibiotic
Staphylococcus aureus	14	20	-	27
Streptococcus pyogenes	14	15	13	20
Streptococcus pneumoniae	16	18	15	25
Enterococcus casseliflavus	-	-	-	24

Table 7
ANTIMICROBIAL ACTIVITY OF TOOTHPASTES WITH AND WITHOUT TINCTURES ON GRAM POSITIVE BACTERIA

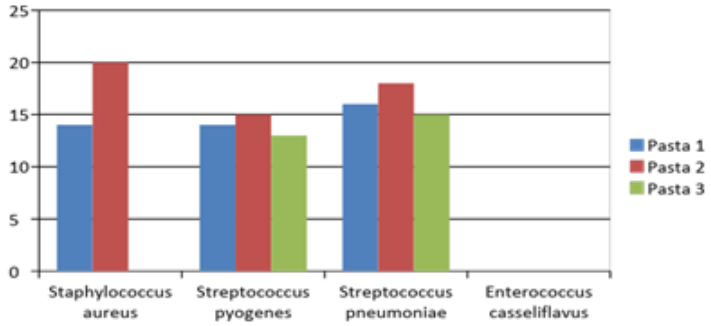

Fig.12 Antimicrobial activity of toothpastes and antibiotic on Gram positive bacteria

Bacterial strains	Toothpaste 1	Toothpaste 2	Toothpaste 3	Antibiotic
Escherichia coli	10	10	-	32
Pseudomonas aeruginosa	15	15	15	27
Enterobacter hormaechei	17	20	15	38

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

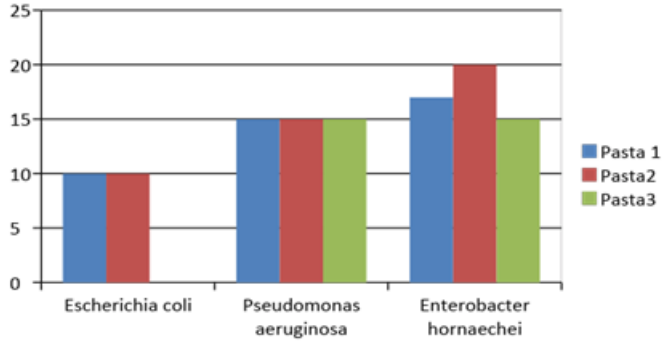

Fig.13. Action of toothpastes and antibiotic on Gram negative bacteria

Fungi	Toothpaste 1	Toothpaste 2	Toothpaste 3	Fluconazole 5µg
Candida albicans	23	24	18	28

Table 9
ANTIMICROBIAL ACTION OF TOOTHPASTES AND ANTIBIOTIC ON FUNGI

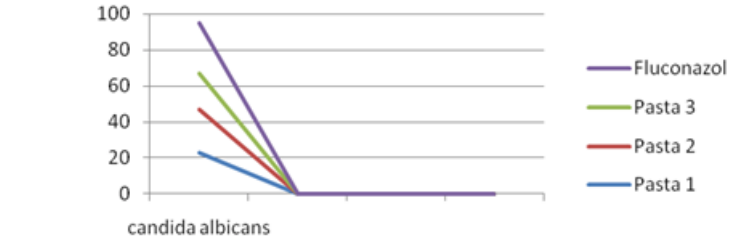

Fig.14. Antimicrobial action of toothpastes on the yeast*Candida albicans*

on*Staphylococcus aureus* and *Enterococcus casseliflavus*, and the results obtained on the other strains showed lower values than tincture toothpastes, hence the benefit in antimicrobial activity shown by added tinctures. The results may be seen in table 7 and figure 12.

The strain Enterobacter hormaechei was affected by the antimicrobial action of Toothpaste 2, which also acted on the other strains, as compared to Toothpaste 1 where lower values were registered. Toothpaste 3 did not show any activity on Escherichia coli, but worked well on the other two strains. The results are shown in table 8 and figure 13.

Table 9 and figure 14 show data on the antimicrobial action of toothpastes on the fungus*Candida candida*. The most effective proved to be Tooth paste 2., and fluconazole was more effective than all toothpastes.

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 Gram positive and Gram negative bacteria, but lower on *Candida albicans.*

The results obtained showed that the alcohol 70° had a weak antimicrobial action on bacterial strains, and the addition of propolis and basil intensified the antimicrobial action, which confirms the antimicrobial properties mentioned in specialized literature.

The mixture of prepared tinctures had superior inhibition capacity, especially on the fungus *Candida albicans* and the bacteria *Streptococcus pyogenes* (24 mm) 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 microbioma, 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.

References

1.SHUAI HUANG, KAI WANG .,- Recent Advances in the Chemical Composition of Propolis.,2014., ISSN 1420-3049 www.mdpi.com/journal/molecules

2. ROBU S., CHESARU B.I, DIACONU C., DUMITRIU B.O., TUTUNARU D, STANESCU U, LISA E.L.,Lavandula hybrida: microscopic characterization and the evaluation of the essential oil Farmacia, 2016, vol. 64, 6.914-917

3. DRAGANESCU M, IANCU A.V., FIRESCU D, DUMITRIU B.O., DIACONU C, REBEGEA L, Trends in antimicrobials consumption and antimicrobial resistance in an infectious diseases hospital from the south-eastern,Farmacia,2016,Vol 64,5,pag.770-774

4.ISLA MI, DANTUR Y, SALAS A, DANERT C, ZAMPINI C, ARIAS M, ORDONEZ R, MALDONADO L, BEDASCARRASBURE E, NIEVA MORENO MI.Effect of seasonality on chemical composition and antibacterial and anticandida activities of Argentine propolis. Design of a topical formulation.Nat Prod Commun. 2012 Oct;7(10):1315-8

5.DUMITRIU BUZIA, O., Tehnologiefarmaceutica, Note de curs, 2015;

6.DUMITRIU BUZIA, O., Tehnologiefarmaceuticã – EdituraZigotto, Galati, 2014 pg 266-275

7.DUMITRIU, B.O., MARDAREN ,DIACONU, C., The Study of Nystatin Release from Microcapsules Obtained by Ionotropic Gelation, Rev. Chim.(Bucharest), **67**, no.2, 2016, 232-235

8.DUMITRIU, B.O., DIMA, C., DIMA, S., Preparation and characterization of chitosan microspheres for vancomycin delivery, Farmacia, 2015, vol. 63, 6,p.897-902

9.DUMITRIU, B.O., DIMA, S., Biopolymer-based Techniques for Encapsulation of Phytochemicals Bioactive in Food and Drug, Mat.Plast.**, 53**,no.1,2016,126-129

10.DUMITRIU, B.O, FASIE, V., MARDARE, N., DIACONU, C., GURAU, G.,TATU, AL,.Formulation,Preparation, Physico-chemical Analysis, Microbiological Peculiarities and Therapeutic Challenges of Extractive Solution of Kombucha. Rev.Chim (Bucharest),**69**, no.3, 2018, p.720-24

11.DRAGANESCU, M., BAROIU, N., BAROIU, L.,DIACONU, C.,DUMITRIU, B.O., Efficient administration of human albumin in clostridium Difficileinfection, Rev.Chim.(Bucharest), **68**, no. 3 2017, p .602-604

12.TATU AL, NWABUDIKE LC. Bullous Reactions Associated With COX-2 Inhibitors Am J Ther. 2017; 24(4):e477-e480.

13.CIOBOTARU O.R, VOINESCU D.C, BARNA O, BARNA I, CIOBOTARU O.C. Influence of the type of anesthesia used, the diet and the consumption of sugar and alcohol on the intradermal skin test to morphine. Biotechnology & Biotechnological Equipment. 2015. 29:5,935-941.

14. TATU AL-Topical Steroid Induced Facial Rosaceiform Dermatitis Acta Endo (Buc) 2016 12: 232-233.

15.CIOBOTARU O.C, CIOBOTARU O.R, VOICU DR, BARNA O, BARNA I, VOINESCU DC. Postoperative pain after total abdominal hysterectomy and bilateral salpingooophorectomy depending on the type of anesthesia administration. Biotechnology &Biotechnological Equipment. 2016.30; 2: 341-345.

16.TATU AL, IONESCU MA, NWABUDIKE LC Contact allergy to topical mometasone furoate confirmed by rechallenge and patch test. Am J Ther. 2018;25(4):e497-e498 2017. 21. Tatu AL, Nwabudike LC. Metoprolol-associated onset of psoriatic arthropathy. Am J Ther.2017; 24(3); e370-e371

17. BRANISTEANU DE, DIMITRIU A, VIERIU M, BODA D, STOLERIU G, MOLODOI DA, BRANISTEANU D. Cutaneous manifestations associated with thyroid disease. Rev Med ChirSoc Med Nat Iasi, 2014; 118(4): 953-958

18. NWABUDIKE, LC, TATU, AL , Response to - Chronic exposure to tetracyclines and subsequent diagnosis for non-melanoma skin cancer in a large Mid-Western US population. J Eur Acad Dermatol Venereol. 2018 ;32(4):e 159

19.GHEORGHE I,TATU AL,LUPU I, THAMER O ,COTAR AI,PIRCALABIORU GG,POPA M,CRISTEA VC,LAZAR V,CHIFIRIUC MC. Molecular characterization of virulence and resistance features in Staphylococcus aureus clinical strains isolated from cutaneous lesions in patients with drug adverse reactions. Rom Biotech Lett. 2017;22(1):12321-27

20.TATU, AL., CIOBOTARU, O.R., MIULESCU, M., DUMITRIU BUZIA O., ELISEI, A.M.,MARDARE, N., DIACONU, C., ROBU, S., NWABUDIKE L, C.,Hydro-chlorothiazide: Chemical Structure, Therapeutic, Phototoxic and CarcinogeneticEffects in Dermatology. Rev.Chim. (Bucharest), **69**, no.8, 2018, p.2110-2114

21. NWABUDIKE, L.C., ELISEI, A.M., BUZIA, O.D., MIULESCU, M., TATU, AL,. Statins. A Review on Structural Perspectives, Adverse Reactions and Relations with Non-melanoma Skin Cancer Rev. Chim. (Bucharest), **69**, no.9, 2018, p.2557-2562

22. TATU AL, NWABUDIKE LC. The Treatment Options of Male Genital Lichen Sclerosus et Atrophicus: Treatments of Genital Lichen SclerosusConference: 14th National Congress of Urogynecology (Urogyn) Location: Eforie, Romania Date: Sep 07-09, 2017 Proceedings Of The 14th National Congress Of Urogynecology And The National Conference Of The Romanian Association For The Study Of Pain 2017 Pages: 262-264

23.BRANISTEANU DE, PINTILIE A, DIMITRIU A,CERBU A,CIOBANU D,OANTA A,TATU AL. Clinical, laboratory and therapeutic profile of lichen planus. The Medical-Surgical Journal 2017 ;121(1):25-32

24. TATU AL,IONESCU MA. Multiple autoimmune syndrome type III-thyroiditis,vitiligo and alopecia areata .Acta Endo (Buc)2017, 13 (1): 124-125

25.TATU AL. Clinico dermoscopic correlations observed in a rosacea group of patients J Am Acad Dermatol 2016.74;(5)suppl 1:AB 104,2713

26.TATU AL,NWABUDIKE LC. Male genital lichen sclerosus—a permanent therapeutic challenge.J Am Acad Dermatol. 2018; 79(3)Supplm 1,AB185

27.TATU AL. Trichoscopic patterns of Adult Alopecia Areata.J Invest Dermatol.2010.130;Suppl 2:S57

28.TATU AL, CLATICI V, Some correlations between the clinical and dermoscopic features of steroid induced facial dermatitis. J Am Acad Dermatol 2015.72;(5)Suppl 1:AB91
29.TATU AL, NWABUDIKE LC. Reply to Happle R. And al. Koebner's sheep in Wolf 's clothing: does the isotopic response exist as a distinct phenomenon?. J Eur Acad Dermatol Venereol. 2018 Feb 28. doi: 10.1111/jdv.14900
30.TATU AL,CRISTEA VC**.** Unilateral Blepharitis with Fine Follicular Scaling. J Cutan Med Surg. 2017; 21(5):442
31. TATU AL,CRISTEA VC.PityriasisFolliculorum of the BackThoracic Area: Pityrosporum, Keratin Plugs, or Demodex Involved?JCutan Med Surg. 2017 ;21(5):441.
32.TATU AL.Nasal spinulosis.JCutan Med Surg.2017;21(3);252
33.TATU AL, NWABUDIKE LC. Reply to: Kubiak K. and al. Endosymbiosis and its significance in dermatology.J Eur Acad Dermatol Venereol.2018 Mar 10. DOI: 10.1111/jdv.14921
34. TATU AL, IONESCU MA, CLATICI VG, et al.Bacillus cereus strain isolated from Demodexfolliculorum in patients with topical steroid-induced rosaceiform facial dermatitis.AnBras Dermatol. 2016; 91:676-7
35. TATU AL, CLATICI V, CRISTEA V.Isolation of Bacillus simplex strain from Demodexfolliculorum and observations about Demodicosisspinulosa.ClinExpDermatol. 2016; 41:818-20
36.TATU AL, IONESCU M A, CRISTEA V C. Demodex folliculorum associated Bacillus pumilus in lesional areas in rosacea. Indian J DermatolVenereolLeprol 2017; 83:610-1
37.TATU AL, NWABUDIKELC .Rosacea-like demodicosis (but not primary demodicosis) and papulo pustular rosacea may be two phenotypes of the same disease-a microbioma, therapeutic and diagnostic tools perspective. J Eur Acad Dermatol Venereol2018 doi: 10.1111/jdv.15166
38.BUZIA, O.D., MARDARE, N.,FLOREA, A., DIACONU, C.,DINICA, R.M., TATU, AL,.Formulation and Preparation of Pharmaceuticals with Anti-rheumatic Effect using the Active Principles of Capsicum Annuum and Piper Nigrum. Rev.Chim.(Bucharest), **69**, no. 10, 2018, p. 2854-2857
39.TATU AL.Skin tags and pregnancy Australas J Dermatol.2010;51 Suppl S1:A42
40.TATU AL The skin and nevi pigmentation during pregnancy. J Am Acad Dermatol.2012.66;(2)Suppl 1:AB 148
41. TATU AL. Dermoscopic structural changes of nevi during pregnancy related to location.J Am Acad Dermatol.2011.64;(2),Suppl1:AB75
42. MIHAILA B, DINICA RM, TATU AL, BUZIA OD. New insights in vitiligo treatments using bioactive compounds from Piper nigrum. Exp Ther Med.2018. doi.org/10.3892/etm.2018.6977

Manuscript received: 15.07.2018