MDRCBB 30th Anniversary Global Symposium

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Innovative probiotics formulations for stabilization of oral microbiome.

Associate Prof. Khaled Nour, Ain-Shams University

Cairo, Egypt

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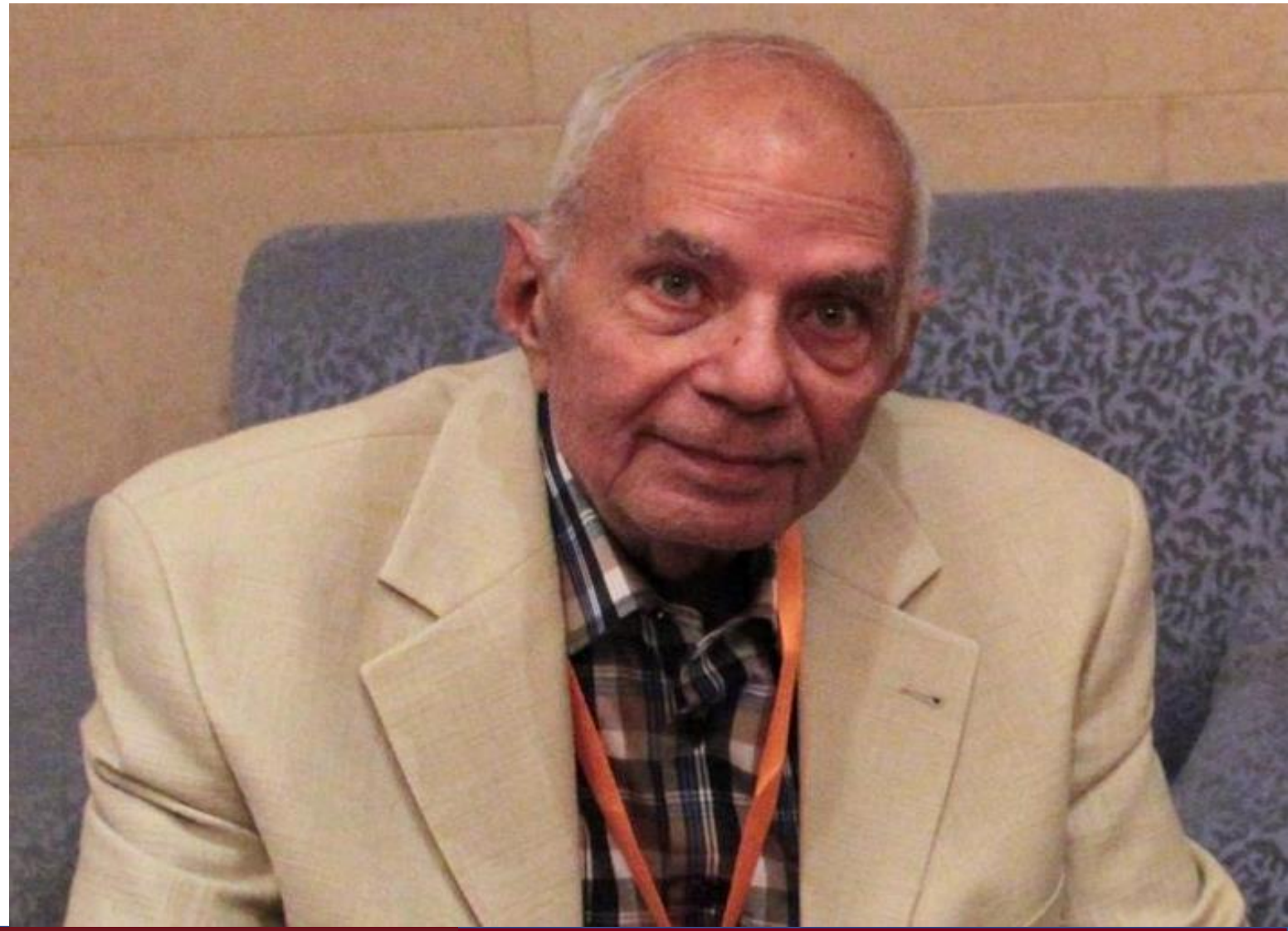
Our Guru...

Prof. Mokhtar Nagy

Professor of Operative Dentistry
Former Dean, Faculty of Dentistry
Ain-Shams University, Cairo Egypt

1946 - 2017

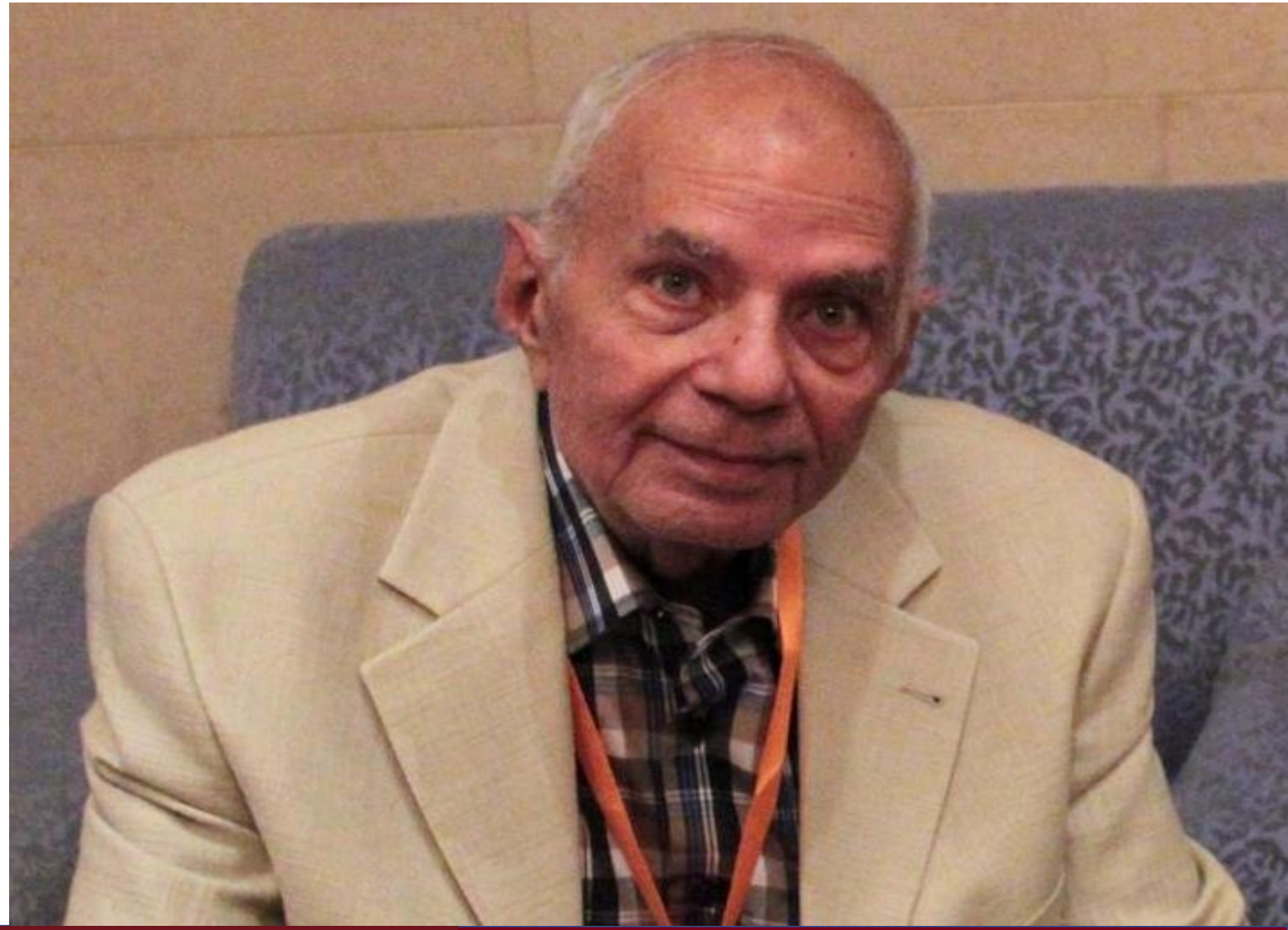
May his soul rest in peace



Our Vision... 2011

Public remedy for oral diseases that is

- Effective
- Sustainable
- Natural ingredients
- Commonly available
- In-expensive



Our Team... 2012

National Research Center

Dr. Hanaa El-Gamily …… Operative Dentistry Department

Prof. Mohamed Zaazou ..… Restorative Dentistry Department

Prof. Nyera Mehanna .…… Food Industries Department

Dr. Shaimaa Nagi ……..... Dental Bio-materials Department

Dr. Ahmed Kassem ……. Pharmaceuticals Department

Ain-Shams University

A. Prof. Khaled Nour ……. Operative Dentistry Dep.

Innovative probiotics formulations for stabilization of oral microbiome

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Our Team... 2012 - 2022

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Dr. Shaimaa Nagi ……..... Dental Bio-materials Department

Dr. Ahmed Kassem ……. Pharmaceuticals Department

Dr. Osama Mosallam ……. Restorative Research Department

Dr. Hoda El-Sayed ……... Dairy Science Department

Dr. Samah El-Sayed ……... Food Science Department

Dr. Sherine Nasry ……..... Oral Medicine Department

Dr. Yousra Aly ……..…… Nutrition Department

Dr. Ahmed Youssef ……..…….Packaging Materials Department

Cairo University

Dr. Rania Mosallam ….. Conservative Dentistry Dep.

Dr. Ibrahim El-Refai ….. Periodontology Dep.

Ain-Shams University

Prof. Muhamad Mokhtar Nagy…….. Endodontic Dep.

A. Prof. Khaled Nour …….. Operative Dentistry Dep.

Innovative probiotics formulations for stabilization of oral microbiome

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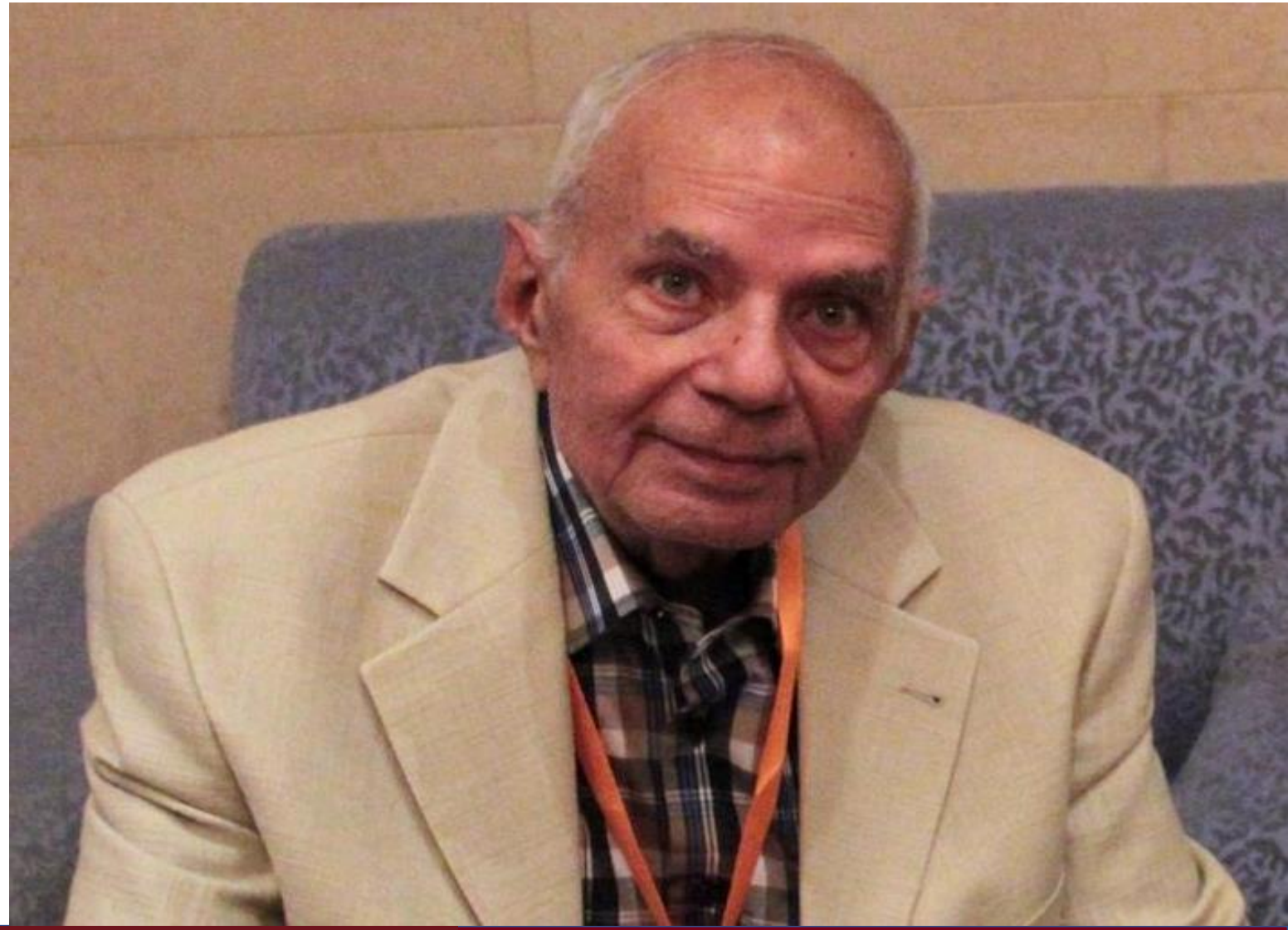
Our Mision... 2012 - 2014

1. Antimicrobial effect of 2 medicinal plant extracts and 1 probiotic strain
2. Determine the minimum inhibitory concentration for each
3. Determine the most effective mixture of the 3 ingredients
4. Test the survival profile of the probiotic strain in this mixture
5. Determine the Cytotoxicity (safety profile) of the mixture
6. Test **In-Vivo** the effect of using



Miswak Extract…

***Sticks from Salvadora persica tree native to India used to brush the teeth in the* Islamic hygienical practices.**

Common, available and in-expensive.

Proven medical benefits and antibacterial effects.

*Al lafi T, Ababneh H (1995). "The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria". International Dental Journal. **45** (3): 218–222*

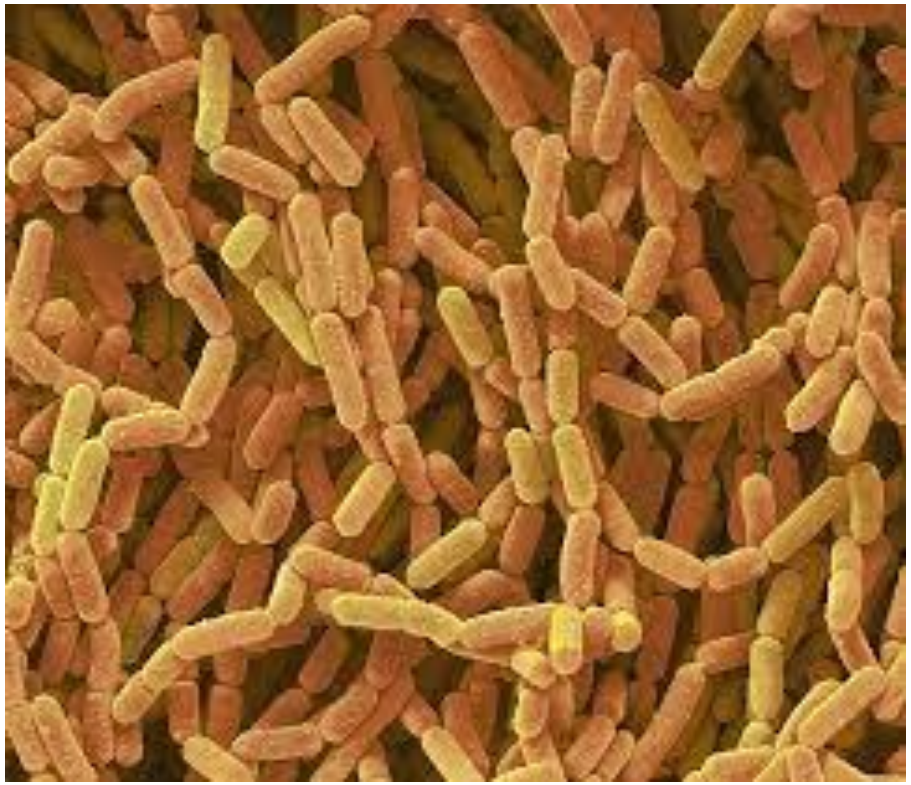
Propolis Extract…

Produced by honey-bees to sterilize their hives.

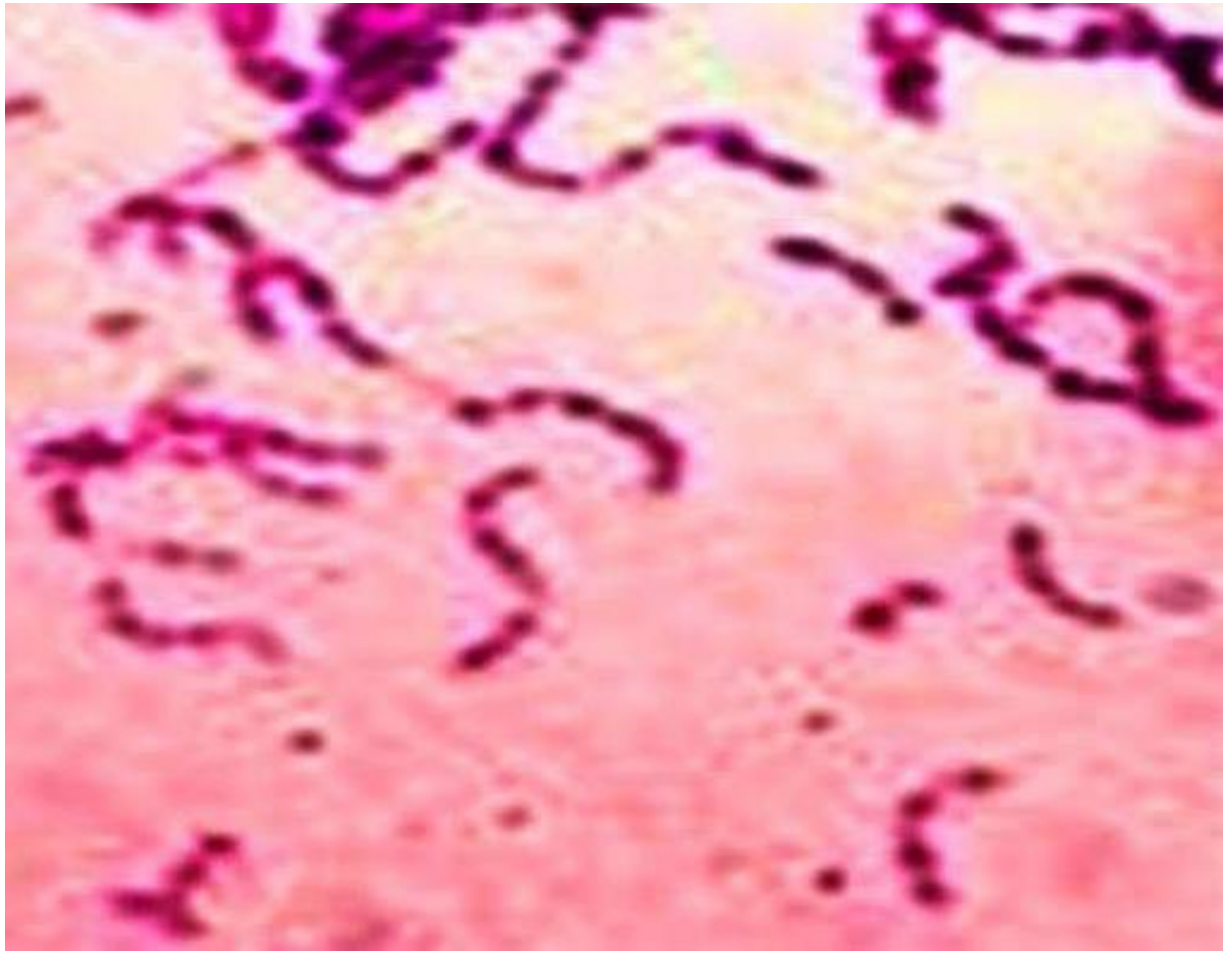
Available, common, proven antimicrobial effect.

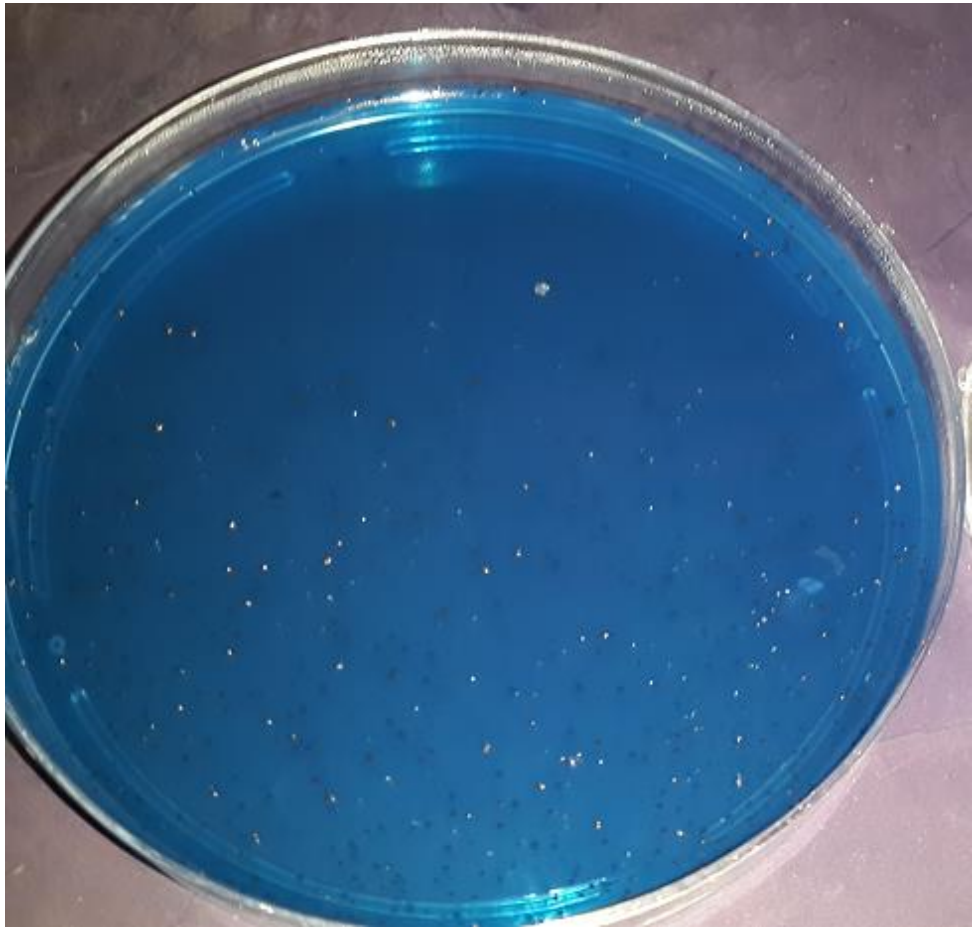
Probiotic Strain…

Lactobacillus Rhamnosus



Isolation of mutans streptococci from swaps from high caries risk individuals for disc diffusion tests





Identification of S. mutans on Mitis salivarius-bacitracin selective medium

14 Toothpastes…

Step 1: *Inert Base*

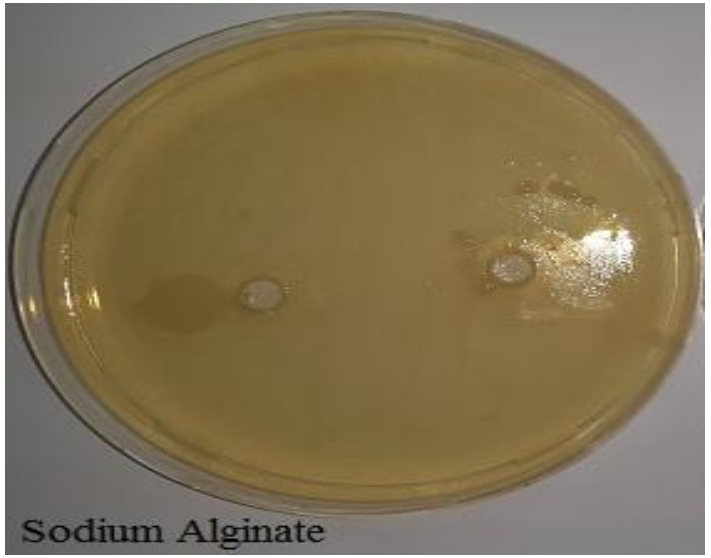
Step 2: *Miswak extract in 3 concentrations 5% , 10% and 20%*
Propolis extract in 3 concentrations 5% , 10% and 20%
Probiotic strain in 3 concentrations 5% , 10% and 20 %

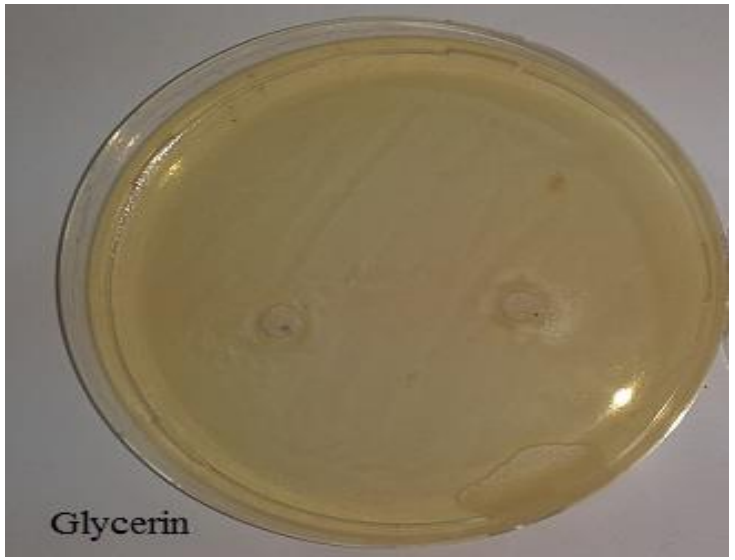
Step 3: Propolis / Probiotic
Miswak / Probiotic
Miswak / Propolis
Miswak / Propolis / Probiotic

Production of an Innert toothpaste BASE

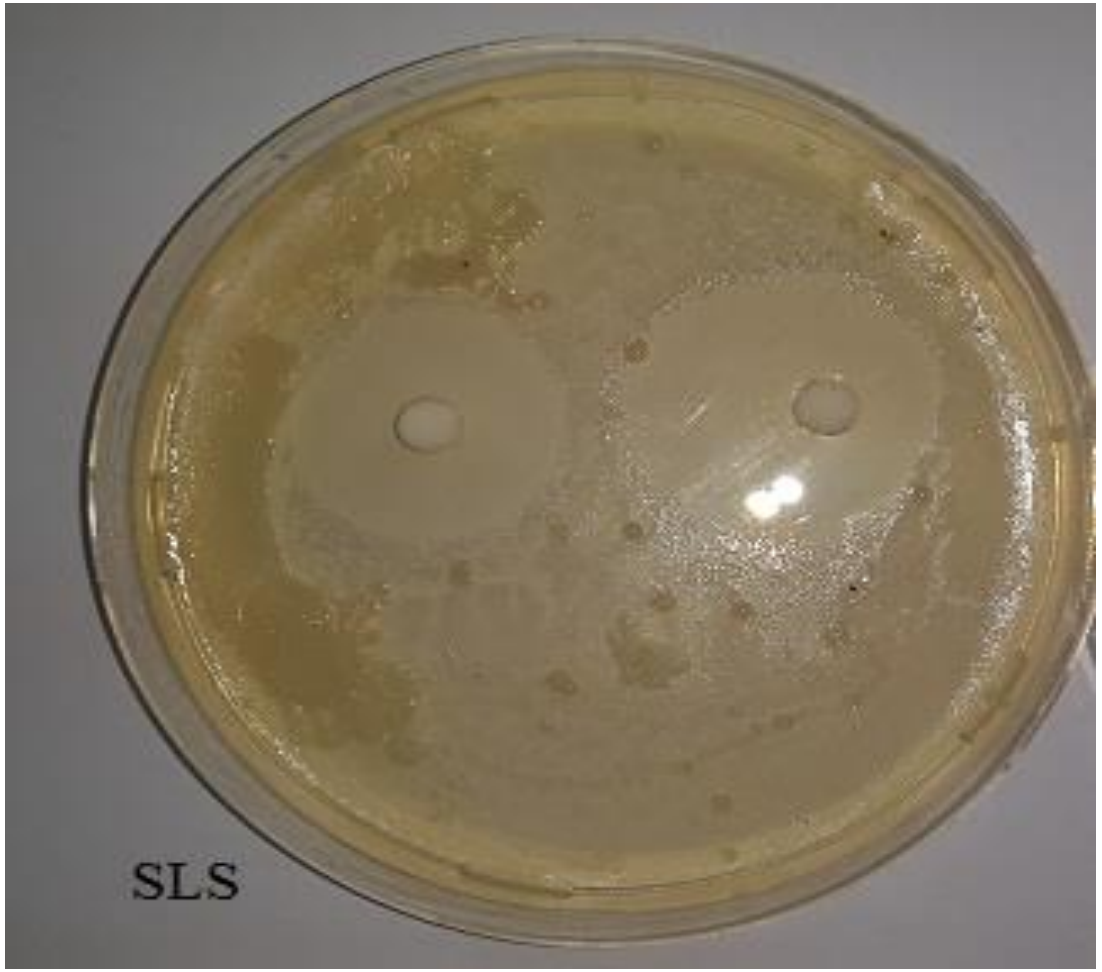
Ingredients	Description	Manufacturer	Qty. Used (%)
Sodium Alginate (C6 H7 O6 Na)	Binding agent	Qualikems Fine Chemicals Pvt.Ltd.,New Delhi- 11060.	25%
Glycerin	Moisturizer	El Gomhouria Co., Cairo, Egypt- B0072GME3L	20%
Sodium lauryl sulfate (C12 H25 O4 SNa)	Foaming agent	S D Fine-Chem Limited (SDFCL)., India.	3% (Replaced)
Peppermint oil	Flavoring agent	FREY+LAU GmbH. D-24558 Henstedt-Ulzburg., Germany	0.5%
Purified water	Vehicle	ARAB Co, (MEPACO-MEDIFOOD).,Egypt	sufficient quantity to get paste consistency

Test the passivity of BASE ingredients by well diffusion method





Replacing the foaming agent Sodium Lauryl Sulphate by Coconut Oil



Disc diffusion test The antimicrobial effect of SLS versus the passivity of coconut Oil

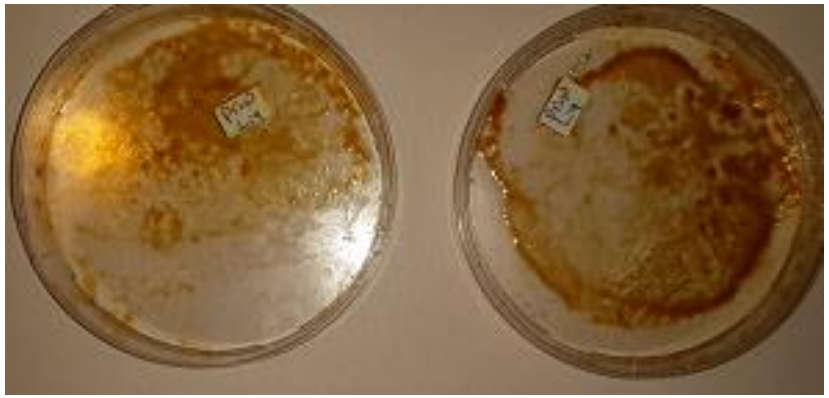
Step 1: Inert Base

Ingredients	Description	Manufacturer	Qty. Used (%)
Sodium Alginate (C6 H7 O6 Na)	Binding agent	Qualikems Fine Chemicals Pvt.Ltd.,New Delhi- 11060.	25%
Glycerin	Moisturizer	El Gomhouria Co., Cairo, Egypt- B0072GME3L	20%
Coconut oil	Foaming agent	Emtenan health shop., Cairo, Egypt.	3%
Peppermint oil	Flavoring agent	FREY+LAU GmbH. D-24558 Henstedt-Ulzburg., Germany	0.5%
Purified water	Vehicle	ARAB Co, (MEPACO-MEDIFOOD).,Egypt	sufficient quantity to get paste consistency

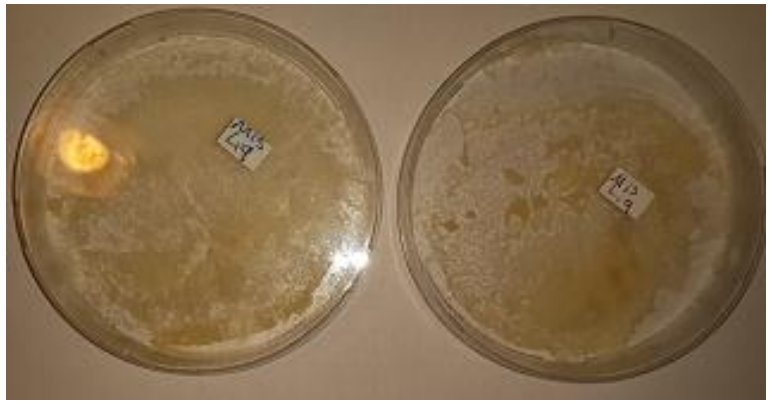
Obtaining 5%, 10% and 20% of the Lyophilized Miswak and Propolis Extracts

Extraction of Miswak and Propolis for 48hrs at 4°c

Supernatants of liquid Miswak extract & Propolis extract



Freeze-dried Propolis extract



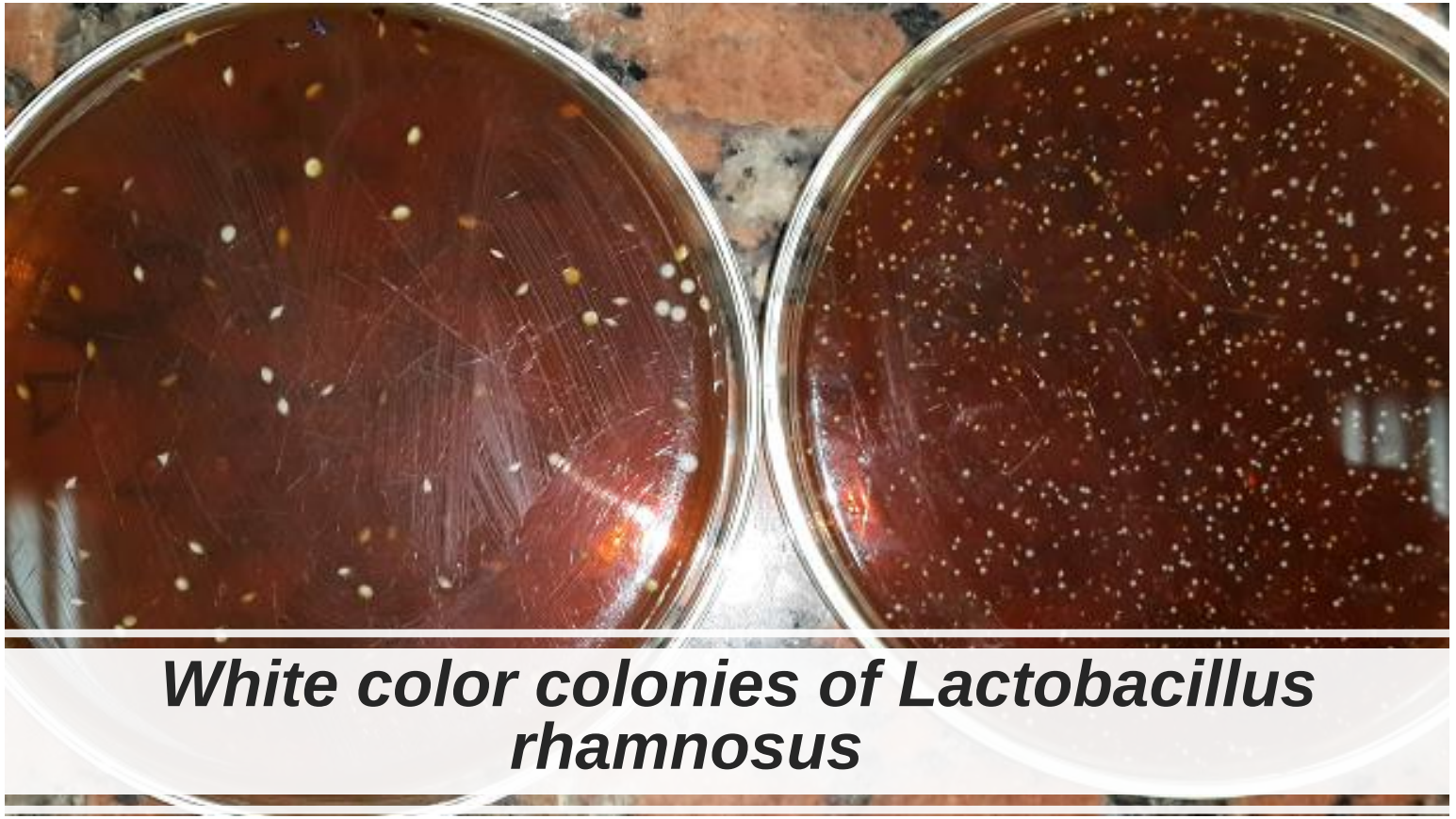
Freeze-dried Miswak extract

Preparation of 5%,10% and 20% concentrations of the Probiotic Strain

Culturing and enumeration of Lactobacillus rhamnosus :
5% Concentration
10% Concentration
20% Concentration

Lyophilyzed Lactobacillus rhamnosus

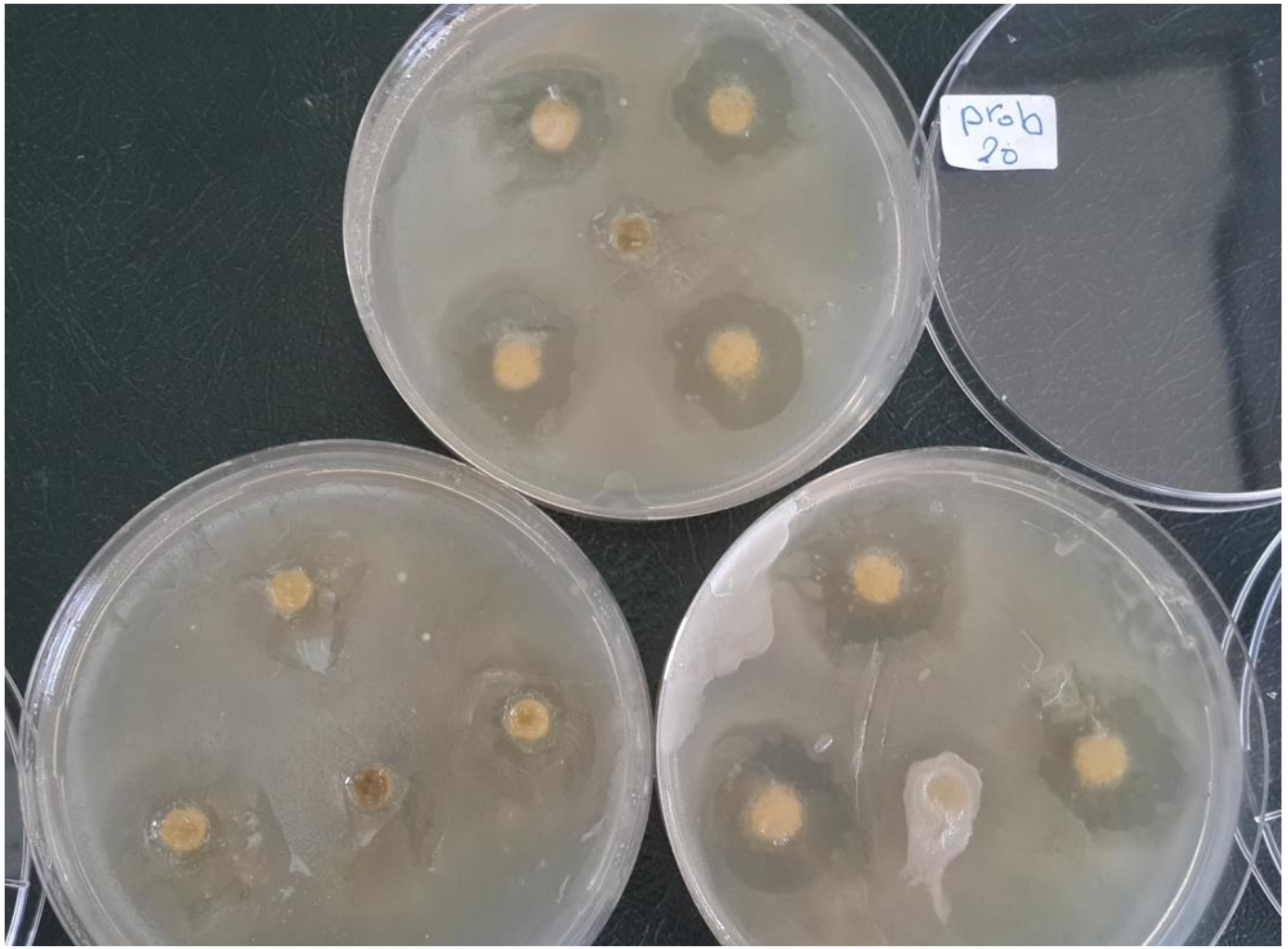
Live cells (CFU/g) = number of colonies ÷ (dilution factor x the amount used to make that pour plate).



White color colonies of Lactobacillus rhamnosus

Toothpastes with different concentrations of the incorporated additives

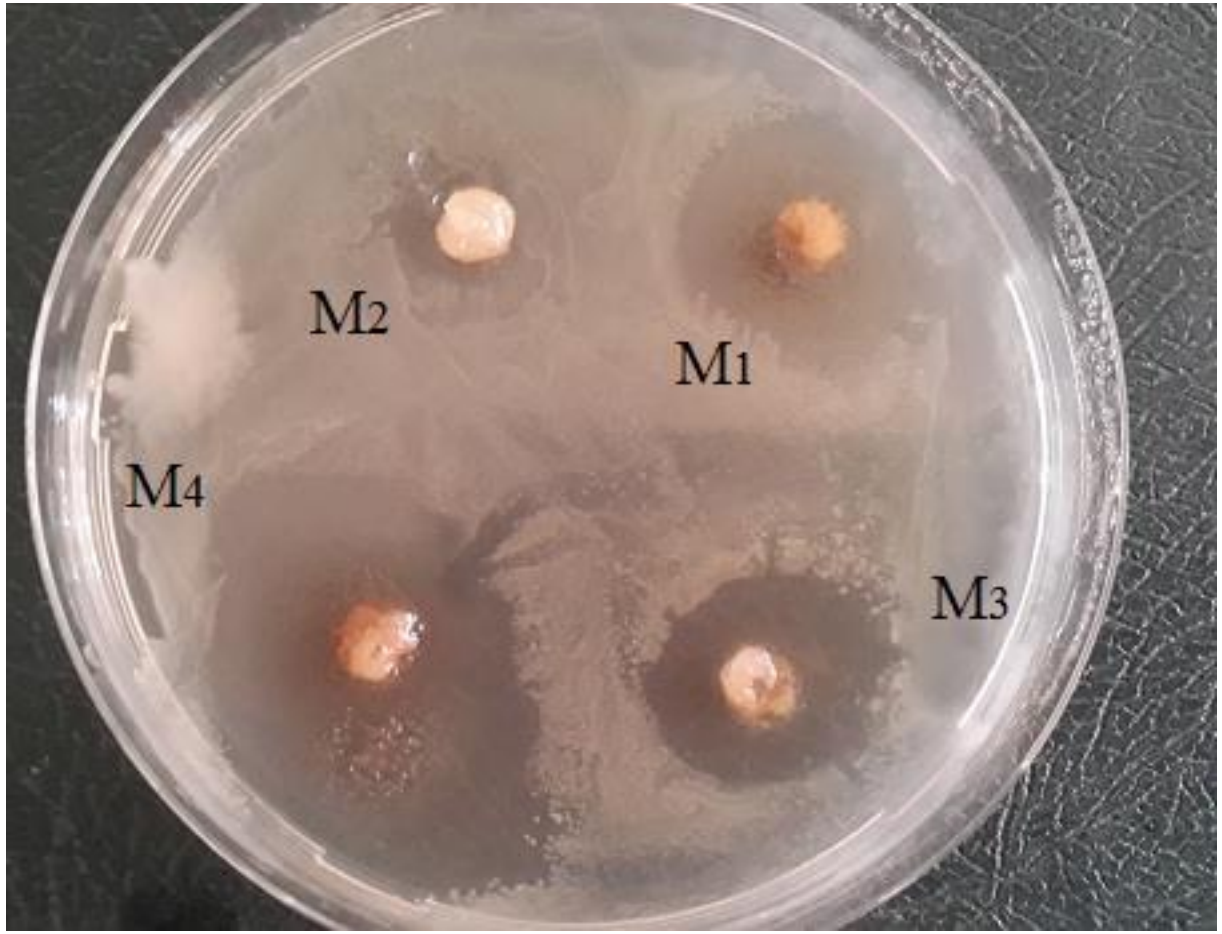
Step 2: Determine the minimum inhibitory concentration for each ingredient



Well diffusion test for 5, 10 and 20% concentration of each ingredient

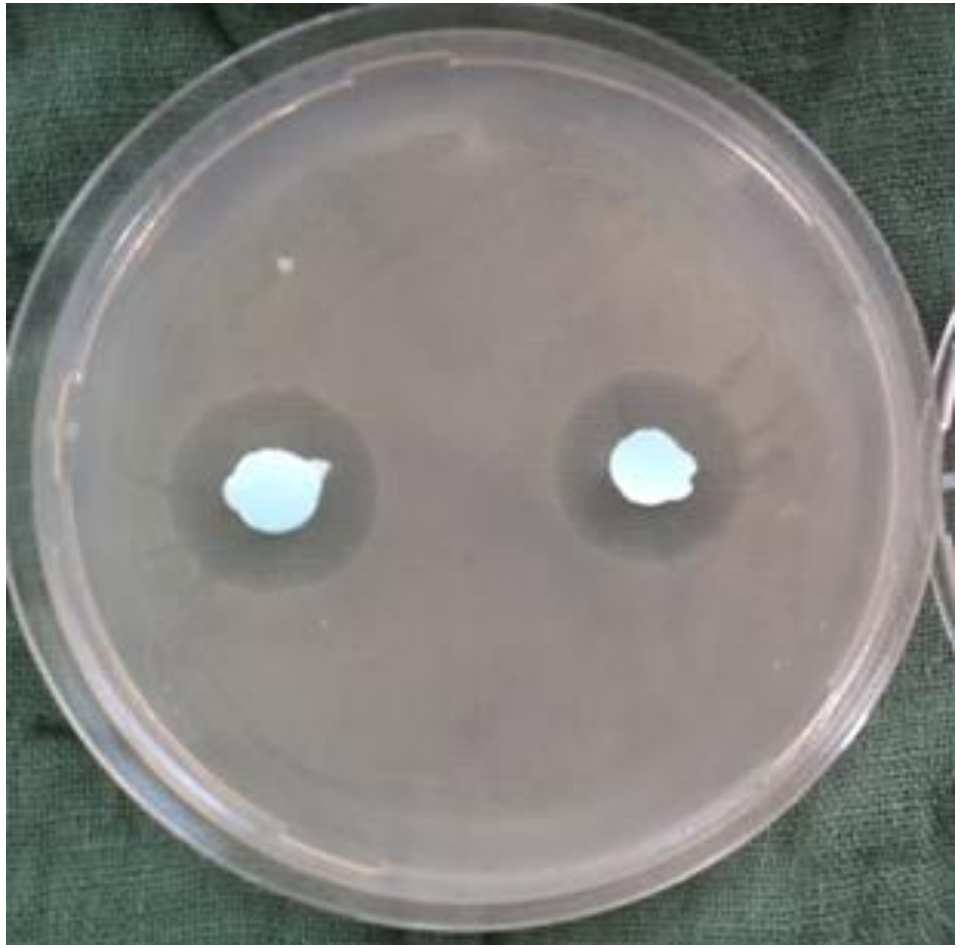
Step 3: Finding the most effective combination of ingredients

M1 : Propolis / Probiotic
M2 : Miswak / Probiotic
M3 : Miswak / Propolis
M4 : Miswak / Propolis / Probiotic

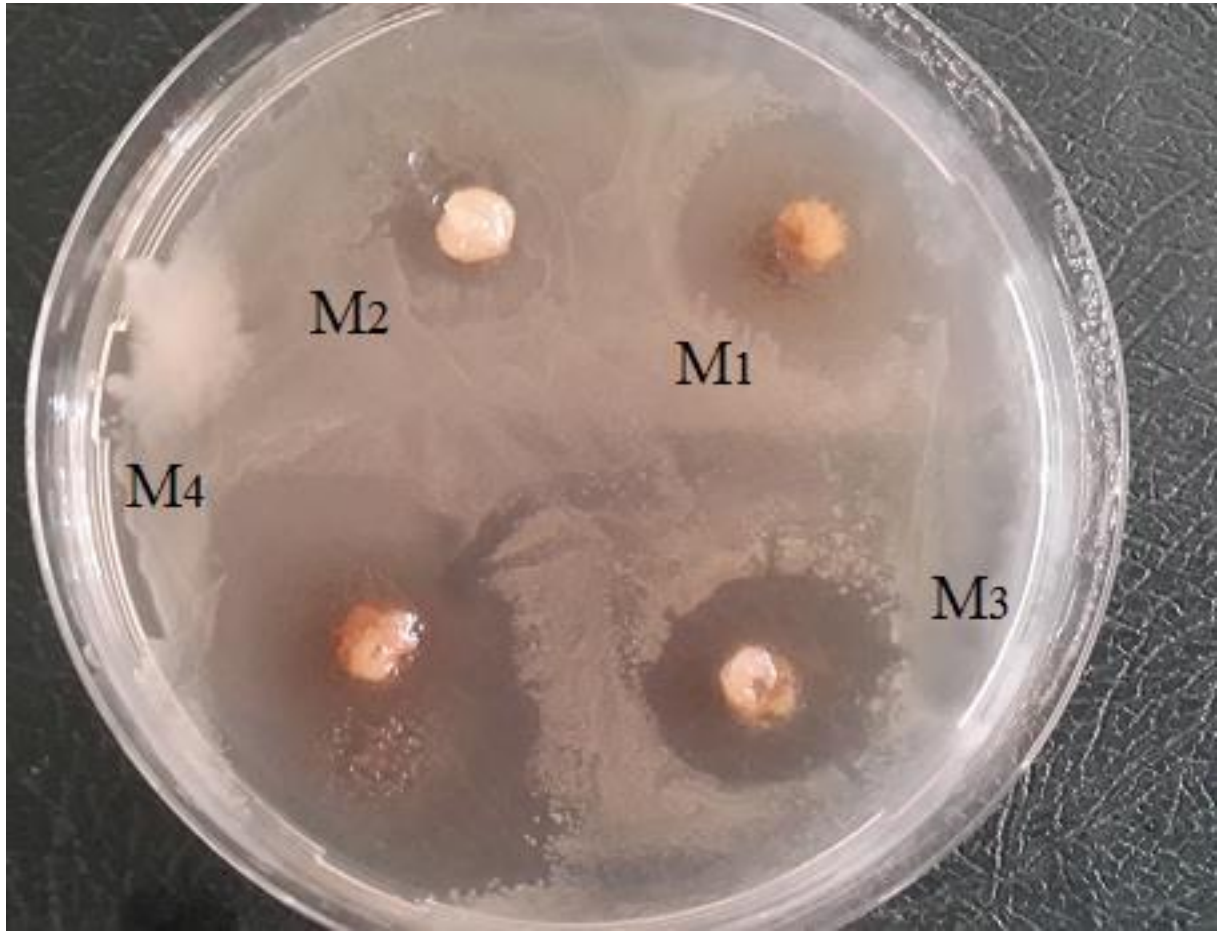


Antimicrobial activity of four experimental toothpastes

Test 1: Comparison with a commercial herbal toothpaste



Antimicrobial activity of commercial herbal toothpaste (Signal Herbal Extracts, Unilever, UK)

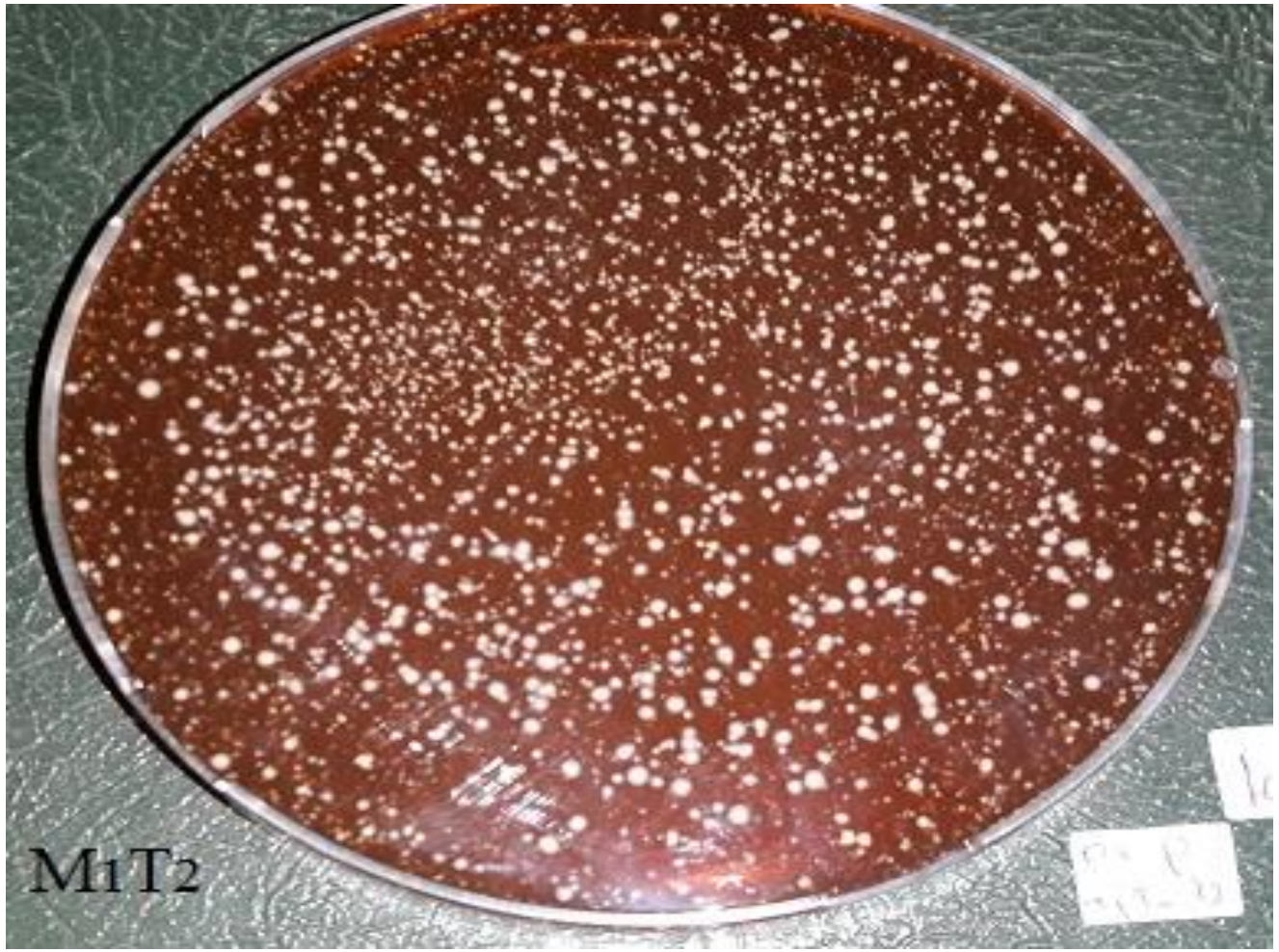


Antimicrobial activity of four experimental toothpastes

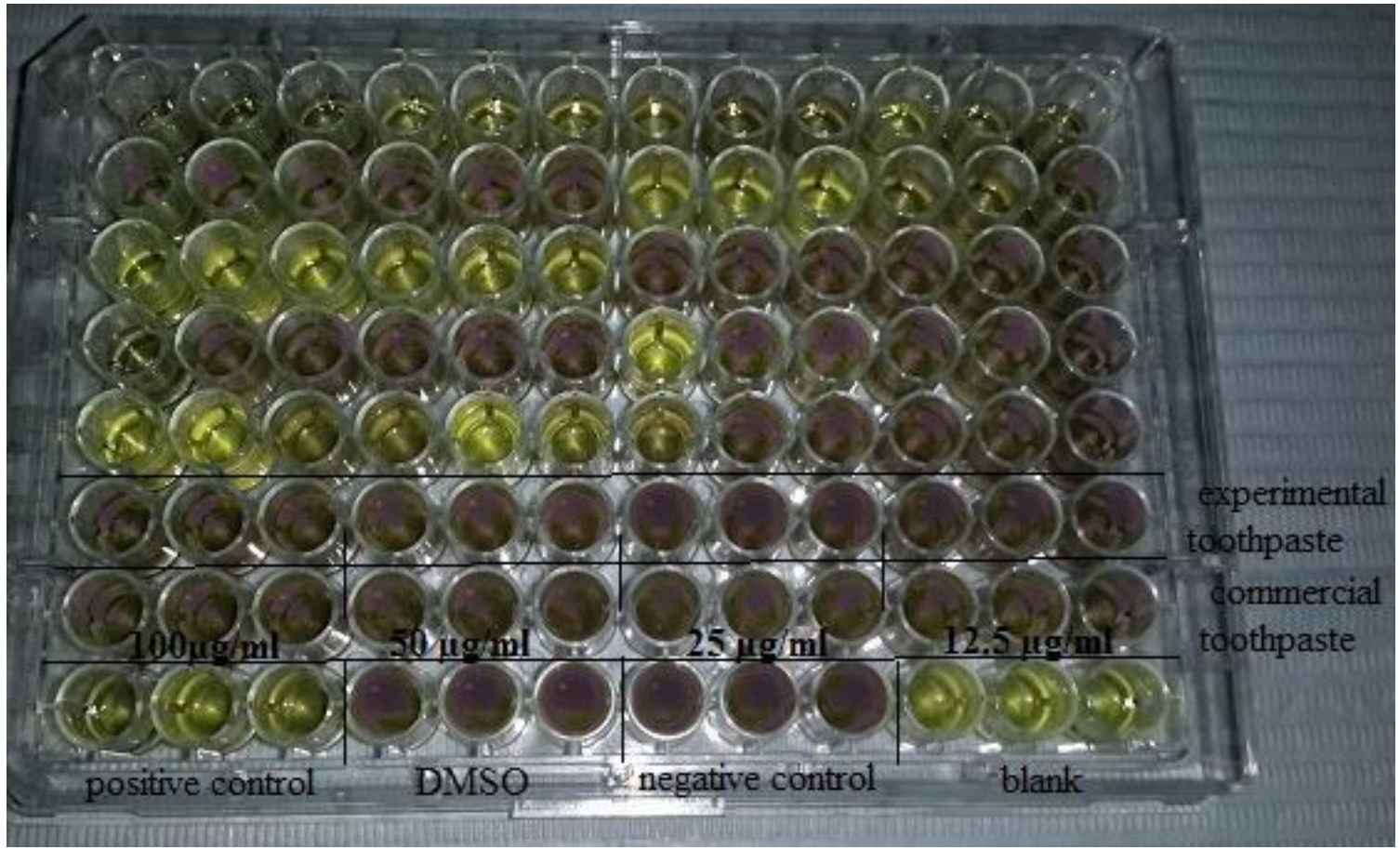
Test 2: Survival profile of the probiotic strain

Colony counting method to test the viability of the probiotic strain with its mixtures upon storage for 15 and 30 days.

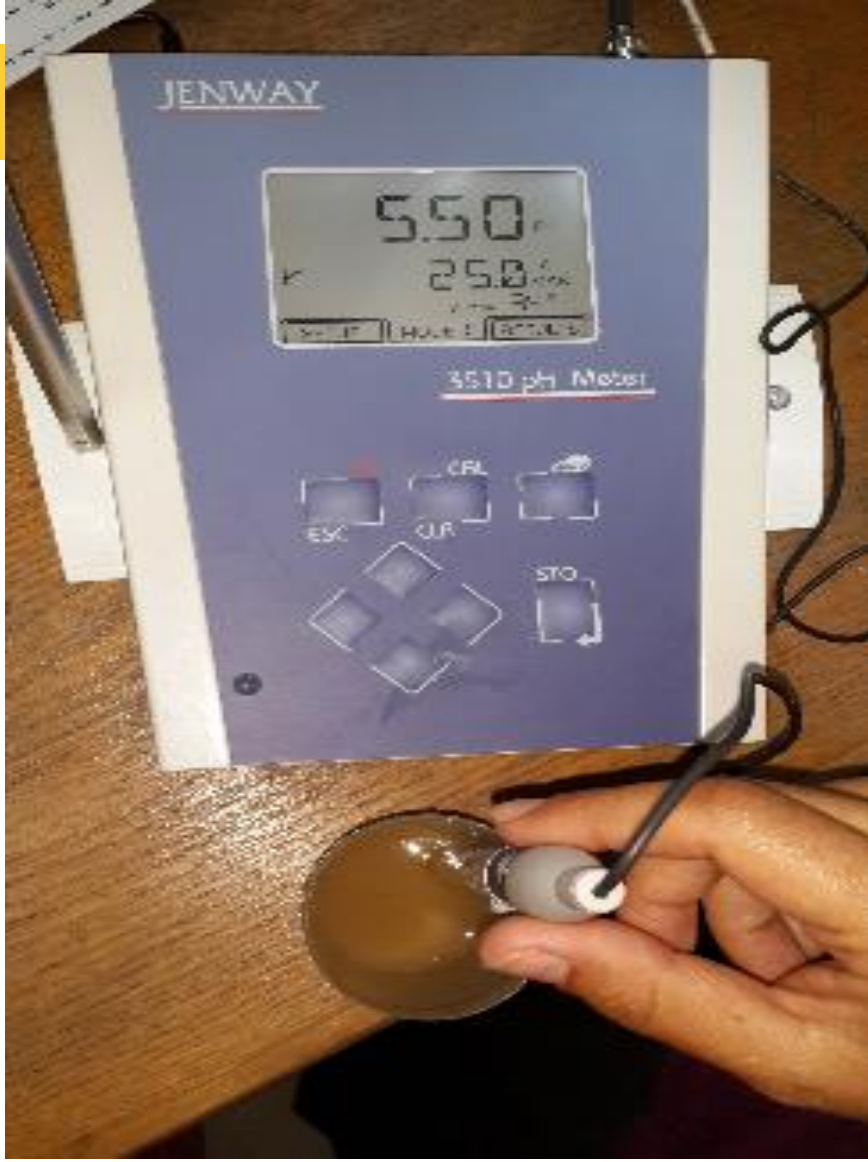
M4 showed the statistically significantly highest mean survival % of L. rhamnosus.



Test 3: pH and Cytotoxicity tests



Cytotoxicity test on Wish cells



pH Meter

pH and Cytotoxicity tests

معمل المعايرات الاحيائية- مزارع الخلايا

المعايرات الاحيائية على خطوط خلايا الاورام البشرية لاستكشاف الادوية

Bioassay–Cell Culture Laboratory

In vitro bioassays on human tumor cell lines for drug discovery

Head: Professor Dr. Bassem El-Menshawi
National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt.
Tel: +20 (2) 3762-1363 Fax: +20 (2) 3336-9603 E-mail: cellculturelabegypt@yahoo.com

Report: Cytotoxic activity test

Sample Code: معجون اسنان تجريبى يحتوى على مركبات طبيعية و بكتريا نافعة
معجون اسنان تجارى

Date:
Submit by: Dr. Hanaa
Received by: DR/ Khaled
The sample was tested against the normal human epithelial cell line:
1- Wish (Normal human epithelial amnion cells)
Sample concentration range between (100 to 0.78 µg/ml) using MTT assay.

Results

Sample Code	LC_{50} (µg/ml)	LC_{90} (µg/ml)	Remarks
معجون اسنان تجريبى يحتوى على مركبات طبيعية و بكتريا نافعة	-------	-------	0% at 100ppm
معجون اسنان تجارى	-------	-------	0% at 100ppm
DMSO	-------	-------	1% at 100ppm
Negative control	-------	-------	0 %

المواد تحت الاختبار امنة على الخلايا الطبيعية للانسان وليس لها ادنى تأثير سمى على الخلايا

LC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs
LC_{90}: Lethal concentration of the sample which causes the death of 90% of cells in 48 hrs
Note: For any publications and/or reporting, please mention that this cytotoxic activity test (*In vitro* bioassay on human tumor cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt.

المركز القومى للبحوث
المشروعات البحثية

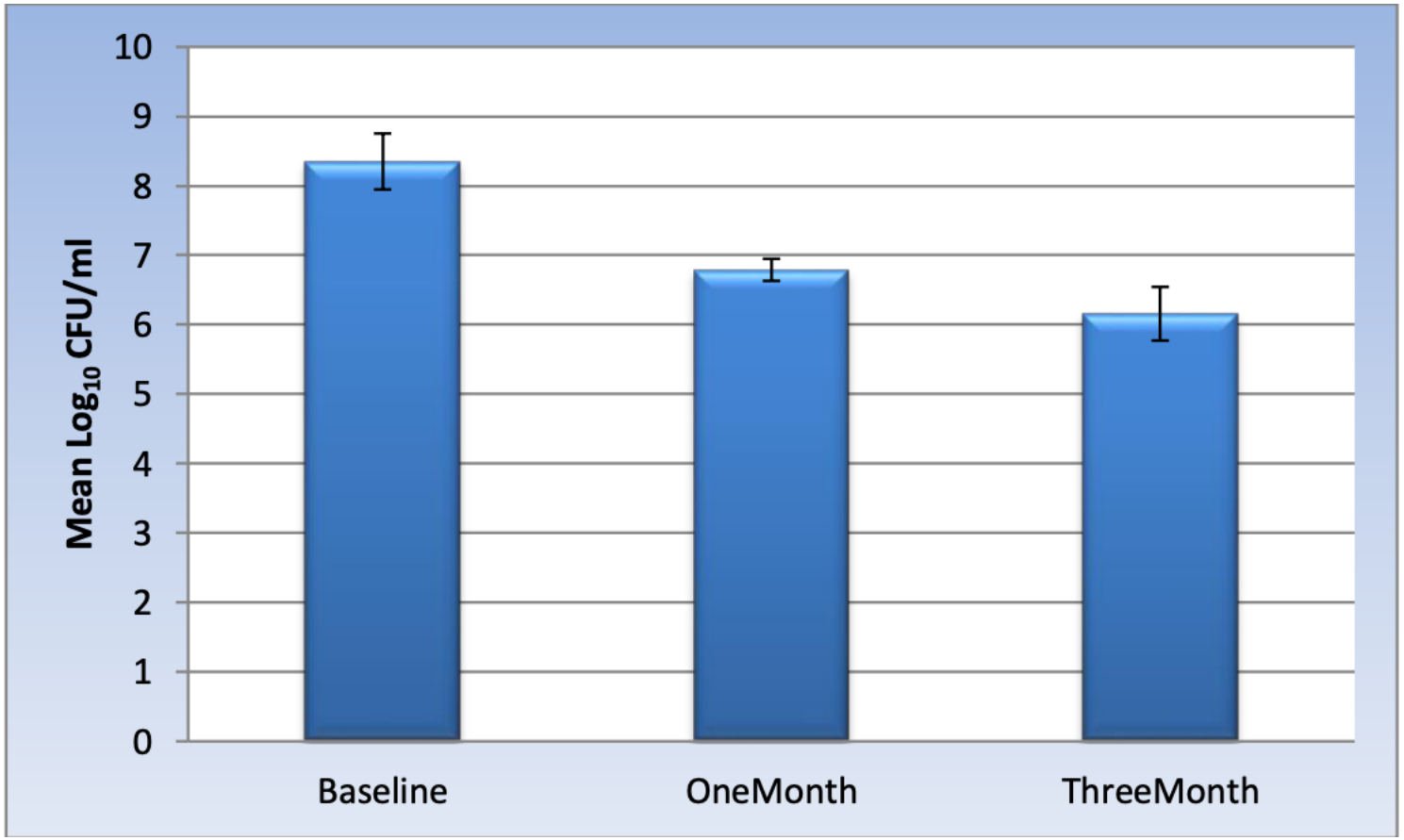
In charge:
Dr. Khaled Mahmoud Mohamed

In-Vivo testing

Salivary mutans streptococal count for high caries risk subjects were monitored for 3 months:

Baseline/ 1month
Highly significant decrease

1 month/3months
Significant decrease



Mean Log 10 CFU/ml of salivary S,mutans at before and after 1 and 3 months of using experimental toothpaste

Patency Pending

Application 2014

Granted 2019

وزارة التعليم العالي والبحث العلمي
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

أكاديمية البحث العلمي والتكنولوجيا
ACADEMY OF SCIENTIFIC RESEARCH AND TECHNOLOGY

براءة إختراع رقم : ٢٩٥٦٩

رئيس أكاديمية البحث العلمي والتكنولوجيا بعد الإطلاع على المادة (١٩) من قانون حماية الملكية الفكرية الصادر بالقانون رقم ٨٢ لسنة ٢٠٠٢، وعلى قرار رئيس الجمهورية رقم ٣٧٧ لسنة ١٩٩٨ بإعادة تنظيم أكاديمية البحث العلمي والتكنولوجيا، وعلى طلب البراءة المقدم تحت رقم ١٨٠٩ فى ٢٠١٤ والمستندات الملحقة به ، قرر:

مادة (١) : تمنح براءة إختراع تحت رقم ٢٩٥٦٩

إلى : المركز القومى للبحوث

المركز العام : ٣٣شارع البحوث - المركزالقومي للبحوث - مكتب اتصال براءات الاختراع – الدقى - جمهورية مصر العربية

عن إختراع تحت مسمى : معجون اسنان يحتوى على تركيبه طبيعية للوقايه من امراض اللثه وتسوس الاسنان.

إسم المخترع : ١- الدكتورة / هناء محمود السيد المهدى الجميلى ٢- الدكتور/ محمد حسين عبد الفتاح زعزوع ٣- الدكتورة/ نايره شاكر مهنا ٤ – الدكتور/ خالد على نور رزق ٥- الدكتورة / شيماء محمد ناجى ٦- الأستاذ الدكتور / أحمد علاء قاسم ٧- الدكتورة / شيرين عادل نصرى يوسف

مـدة البراءة : عشرون عاماً تبدأ من يوم ٢٠١٤/١١/١٠ ، وقد توضح بياناتها في الوثائق المعتمدة المرفقة بهذه الشهادة،

مادة (٢) : صدر هذا القرار بالقاهرة فى ٣١ ديسمبرذ ٢٠١٩

مادة (٣) : على الجهة المختصة نشره فى جريدة براءات الإختراع

رئيس
أكاديمية البحث العلمى والتكنولوجيا
" د.محمود محمد صقر "

قائم بأعمال
رئيس مكتب براءات الاختراع
" د.منى محمد يحيى "

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-Vitro comparative Study of Antimicrobial Activity of Two Plant Extracts and Probiotic Strain against Isolated oral Cariogenic Pathogen.

Hanaa M Elgamily[1*], Shimaa M Nagi[1], Ahmed A Kassem[2], Khaled A Nour[3], Mohamed H Zaazou[1], and Nayra S Mehanna[4].

European Journal of Dentistry, Volume 12 / Issue 1 / January-March 2018

Original Article

Antibacterial effectiveness of probiotic-based experimental mouthwash against cariogenic pathogen: An *in vitro* study

Hanaa Elgamily[1], Osama Mosallam[1], Hoda El-Sayed[2], Rania Mosallam[3]

[1]Restorative and Dental Materials Research Department, Division of Oral and Dental Research, National Research Centre, Giza, Egypt,
[2]Dairy Science Department, Division of Food Industries and Nutrition, National Research Centre, Giza, Egypt,
[3]Department of Conservative Dentistry, Faculty of Oral and Dental Medicine, Cairo University, Giza, Egypt

Correspondence: Dr. Hanaa Elgamily
Email: hanaa_elgamily@yahoo.com

Journal of International Dental and Medical Research ISSN 1309-100X
http://www.ektodermaldisplazi.com/journal.htm

The Clinical Efficacy of A Probiotic Miswak Oral Spray in Patients with Gingivitis

Sherine Nasry[1], Hanaa Elgamily [2*], Ibrahim El-Refai [3], Nayra Mehanna[4]

1. Department of Surgery and Oral Medicine, Oral and Dental Division, National Research Centre, Giza, Egypt.
2. Researcher at restorative and Dental Materials Department, Oral and Dental Division, National Research Centre, Giza, Egypt.
3. Department of Periodontology and Diagnosis, Cairo University, Giza, Egypt.
4. Professor at Department of Dairy Science (Microbiology Lab.), Food Industries and Nutrition Division, National Research Centre, Giza, Egypt.

A Promising Probiotic Irrigant: An *In Vitro* Study

Hoda El-Sayed[1], Yousra Aly[2], Hanaa Elgamily[2], Mohamed M. Nagy[3*]

[1]National Research Centre, Dairy Science Department (Microbiology Lab.), Food Industries and Nutrition Division, Giza, Egypt; [2]National Research Centre, Restorative Dentistry and Dental Material Department, Giza, Egypt; [3]Endodontic Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

CONCLUSION: *Lactobacillus rhamnosus* which revealed a potential inhibitory effect on the growth of *Enterococcus faecalis*, could be used as a new natural, safe probiotic irrigant agent.

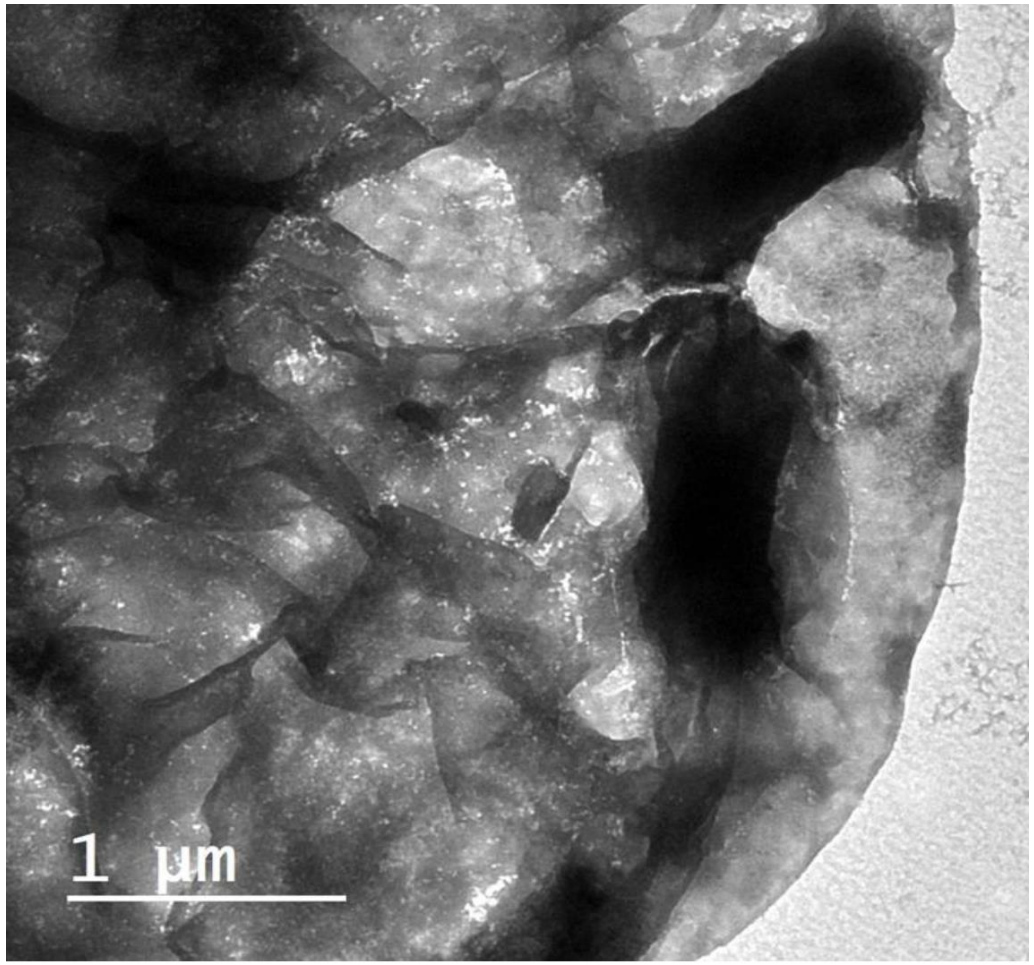
Anti-cariogenic and Remineralization Efficacy of Probiotic Jelly Candy Supplemented with Natural Nano Prebiotic

Hanaa M. Elgamily [1], Samah M. El-Sayed [2], Hoda S. El-Sayed [2], Ahmed M. Youssef [3]

1 Restorative and Dental Materials Department, Oral and Dental Research Institutes, National Research Centre, Giza, Egypt.

2 Dairy Science Department, National Research Centre, Giza, Egypt.

3 Packaging Materials Department, National Research Centre, Giza, Egypt.



TEM of Jelly Candy showing probiotic inside

Conclusion

The nano emulsion of red grape seeds and *Lacticaseibacillus rhamnosus* probiotic strain impregnated in jelly candy showed aanti-cariogenic and remineralizing potential.

Its formulation is likely to come at limited costs and its application may have high cost-effectiveness.

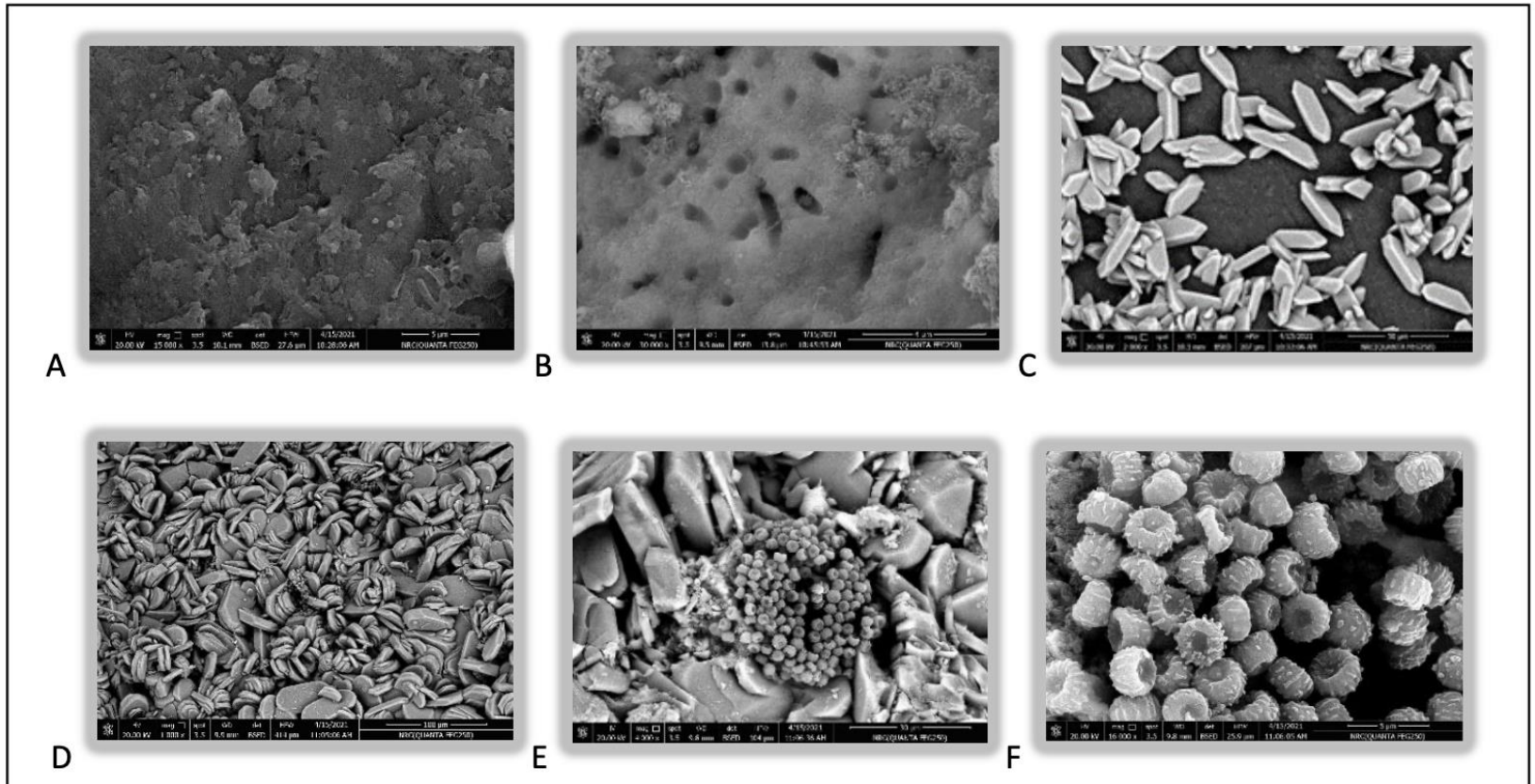


Fig. 3 SEM images of enamel discs showing: (Fig. 3A) 21 days growth of *Streptococcus mutans* (*S. mutans*) and *Actinomyces viscous* (*Actino. Viscus*) without treatment; (Fig. 3B) after 21 days exposure to sterile saline for 10 min twice /day; (Fig. 3C) SEM image of the crystals formed on the enamel surface after using probiotic- GSE nano emulsion-based Jelly candy and (Fig. 3D) was clearly displayed trapping of *S. mutans* in Jelly candy. (Fig. 3E-F) Magnified micrograph of (Fig. 3D) at magnification (4000x), (16000x) respectively.

MDRCBB 30th Anniversary Global Symposium

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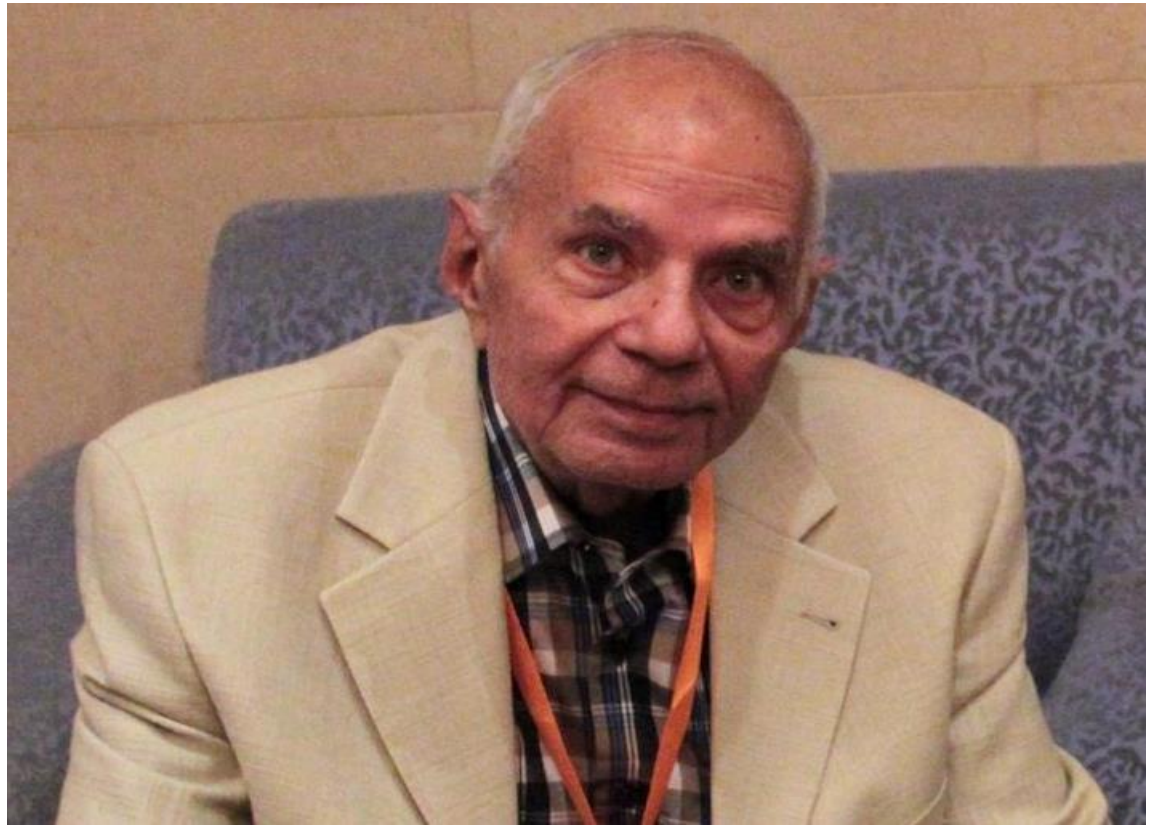
Prof. Mokhtar Nagy

Professor of Operative Dentistry

Former Dean, Faculty of Dentistry
Ain-Shams University, Cairo Egypt

1946 - 2017

May his soul rest in peace



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