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Formulation of a Moisturizing Cream Based on *Leucaena leucocephala* (Lam)Galactomannan harvested in El Oued region(Algeria)

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بسم اللّهِ الرَّحْمَـٰنِ الرَّحِيـم

﴿ فَرِحِينَ بِمَا آتَاهُمُ اللَّهُ مِنْ فَضْلِهِ ﴾

هذه اللحظة لا تورث ولا تشترى، لقد دفعت ثمنها عمري و شبابي، سهري، خوفي، ودموعي، هي لهفة الوصول و دمعة الحزن، فرحة العمر هذه هي النهاية السعيدة لبدايات أعظم و أصبح حلمي حقيقة

أهدي تخرجي إلى كل ما أملك، إلى العمود الثابت في عثرات أيامي، إلى من هي في الحياة حياة، إلى من بذلت جهد السِنين من أجلي " أختي"، إلى من قامت بالدورين، فمهما كتبت من كلمات فلن توفيها حقها، أسأل الله أن يجزيها عني خير الجزاء وأن يديمها نعمة في حياتي.

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رحمكِ الله يا أمي وأسكنكِ فسيح جناته، وجعل هذا النجاح صدقة جارية في ميزان حسناتك

إلى من أكرمني و أعزني

إلى من قيل فيهم، ﴿ سَنَشُدُّ عَضُدَكَ بِأَخِيكَ ﴾

إخوتي، لم أصل لولا دعمكم و عطائكم بلا مقابل، فأنتم قوتي و ملاذي بعد الله، أدامكم الله ضلعاً ثابتاً لي.

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﴿ قَالَ قَدْ أُوتِيتَ سُؤْلَكَ ﴾

بين فكرة وُلدت في عتمة الحيرة، وسطرٍ أخيرٍ خُطَّ بمداد الفرح... مرت أيامٌ قاسينا سهر الليل ، وارهقنا الفكر و الورق فِكرَه. ولم يكن هذا الدرب لِيُطوى لولا قناديلٌ بشرية أضاءت عتمته، وأيدٍ خفية سندت تعبه

أفتحُ قلبي قبل صفحاتي لأهدي هذا الجهد المتواضع إلى:

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" جدتي " نبع البركة والوقار، ودعواتها الطاهرة التي تحرس خطواتي وتمنع الأيام دِفنها

" جدي و جدتي " الجذور الطيبة والأرواح الغالية التي يمتد منها الفضل والخير، ومن يسكنون في عمق القلب والذاكرة.

"عمتي العزيزة"، رمز الطيبة والعطاء.. أهدي إليكِ هذا النجاح تقديراً لم.كانتكِ الغالية في قلبي، ودعمكِ الذي لم ينقطع

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حميده اسماء

Formulation of a Moisturizing Cream Based on *Leucaena leucocephala* Galactomannan

Abstract

This study focused on developing a sustainable oil-in-water (O/W) Moisterized cream using galactomannan extracted from seeds of Leucaena leucocephala collected in El Oued, Algeria. The water-soluble polysaccharides were obtained through hot water maceration at 65°C. The optimized cream was developed by adding 5% GSLL mucilage to the water phase, using Emulgin B2 as emulsifiers. resulting in an extraction yield of 11.66%. Chemical analysis of the crude extract (GSLL) showed a high carbohydrate content of 97.7 ± 0.28% total sugars, composed of 62.98 ± 3.62% neutral sugars, along with 15 ± 0.43% co-extracted proteins and 4.74 ± 0.27% total polyphenols. The final emulsion had a stable, uniform, light beige appearance with enhanced viscosity. It exhibited a skin-friendly pH of 7.16, consistent spreadability (35.65 g/cm²·s), and a measurable intrinsic Sun Protection Factor (SPF) of 9.53. The formulation proved highly resistant to freeze-thaw cycles during stability testing, though it showed phase separation when exposed to intense mechanical or thermal stress. Microbiological evaluation showed fluctuations in the total aerobic mesophilic bacterial count and low fungal contamination during storage. No pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *coagulase-positive Staphylococci*, were detected in any sample, indicating an acceptable microbiological profile of the formulated cream ,according to the national standards. Moreover, the formulated cream exhibited a notable antioxidant capacity with an IC50 value of 1.76±0 mg/mL in the DPPH assay. It also demonstrated promising antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, showing comparable efficacy to gentamicin. overall, galactomannan from Leucaena leucocephala emerges as a highly effective and biocompatible natural stabilizer suitable for dermocosmetic applications.

Keywords:*Leucaena leucocephala*, Galactomannan, Moisterized cream and skin, Microbiological Quality Control , Biological Activities

تحضير كريم مرطب قائم على الجالاكتومانان المستخلص من نبات *Leucaena leucocephala*

الملخص

ركزت هذه الدراسة على تطوير وتقييم مستحلب مرطب مستدام من نوع الزيت في الماء (O/W) باستخدام الجالاكتومانان المستخلص من بذور نبات اللوسينيا البيضاء (Leucaena leucocephala) التي جُمعت من منطقة الوادي في الجزائر. وقد تم الحصول على عديدات السكاريد القابلة للذوبان في الماء عن طريق النقع في الماء الساخن عند درجة حرارة 65 درجة مئوية. تم تطوير الكريم الأمثل بإضافة 5% من صمغ GSLL إلى الطور المائي، باستخدام Emulgin B2 و Cutinaكمستحلبات. مما أسفر عن مردود استخلاص بلغ 11.66%. وأظهر التحليل الكيميائي للمستخلص الخام (GSLL) محتوىً عالياً من الكربوهيدرات بنسبة 97.7 ± 0.28% من إجمالي السكريات، تتكون من 62.98 ± 3.62% من السكريات المتعادلة، بالإضافة إلى 15 ± 0.43% من البروتينات المستخلصة معها، و4.74 ± 0.27% من إجمالي البوليفينولات. تميز المستحلب النهائي بمظهر بيج فاتح ثابت ومتجانس مع لزوجة محسّنة. كما أظهر درجة حموضة مناسبة للبشرة تبلغ 7.16، وقابلية توزيع ثابتة (35.65 جم/سم²•ثانية)، وعامل حماية من الشمس (SPF)قابل للقياس يبلغ 9.53. أثبتت التركيبة مقاومة عالية لدورات التجميد والذوبان خلال اختبارات الثبات، على الرغم من ظهور انفصال طوري عند تعرضها لإجهاد ميكانيكي أو حراري شديد. أظهرت التحاليل الميكروبيولوجية تذبذبًا في تعداد البكتيريا الهوائية المتوسطة المحبة للحرارة وانخفاضًا في التلوث الفطري خلال فترة التخزين. ولم يتم الكشف عن أي بكتيريا ممرضة، بما في ذلك *Escherichia coli* و *Pseudomonas aeruginosa* والمكورات العنقودية موجبة التخثر *coagulase-positive*) (*Staphylococci*، في أي من العينات المحللة، مما يدل على أن الكريم المُحضَّر يتمتع بنتائج ميكروبيولوجية مقبولة استنادا الى المعايير الوطنية.علاوة على ذلك، أظهر الكريم المُصاغ قدرة ملحوظة كمضادللأكسدة بقيمة IC50 بلغت 1.76±0 مغ/مل في اختبار الـ .DPPH كما أظهر نشاطاً واعداً

مضاداً للبكتيريا ضد سلالات *Escherichia coli*و *Staphylococcus aureus*، مسجلاً فعالية مقاربة للمضاد الحيوي الجنتاميسين.(Gentamicin) بناءً على ذلك، يظهر الجالاكتومانان المستخلص من بذور نبات اللوسينيا البيضاء كمثبت طبيعي فعال للغاية ومتوافق حيويًا وملائم للتطبيقات التجميلية والعلاجية للبشرة.

الكلمات المفتاحية:*Leucaena leucocephala*، جالاكتومانان، كريم مرطب للبشره، مراقبة الجودة الميكروبيولوجية، الأنشطة البيولوجية

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Introduction

Introduction

Moisturizing creams are among the most essential cosmetic products in daily skincare routines, as they help improve skin hydration, reinforce the epidermal barrier, and reduce irritation caused by environmental stressors such as cold, heat, and pollutants (Cosmetic Ingredient Review Expert Panel, 2019). In recent years, there has been a growing demand for natural, sustainable, and biocompatible ingredients in cosmetic formulations, which has prompted researchers to explore plant-derived polysaccharides as alternatives to synthetic thickeners and emulsifiers. Among these biopolymers, galactomannans have attracted particular attention due to their gelling, thickening, and moisturizing properties, as well as their favorable safety profile in cosmetic use.

Galactomannan derived from *Leucaena leucocephala* seeds is a natural polysaccharide rich in D-mannose and D-galactose units, with a molar ratio close to 1:1, and a high carbohydrate content that exceeds 70% of the total seed gum (Mittal et al., 2016; Al-Jawadi et al., 2021). This composition confers high water-holding capacity and viscosity, making galactomannan suitable as a gelling and moisturizing agent in topical formulations. Studies on *Leucaena leucocephala* galactomannan have shown that it can form stable colloidal systems and hydrogels, which are promising for use in dermatological and cosmetic products (Mittal & Kaur, 2019; Al-Jawadi et al., 2021).

In this context, the present research entitled “Formulation of a Moisturizing Cream Based on *Leucaena leucocephala* Galactomannan” aims to develop and evaluate a moisturizing cream where seed galactomannan acts as a key thickening and hydrating component. The work focuses on designing an oil-in-water emulsion system, optimizing the formulation , and assessing the physical and rheological properties of the final cream, including viscosity, spreadability, and stability under storage conditions..

This study contributes to the valorization of locally available plant resources and supports the development of eco-friendly cosmetic formulations based on renewable natural polymers. By exploring *Leucaena leucocephala* galactomannan in a moisturizing cream , our memoir is structured into four chapters. The first chapter focuses on galactomannan sugar, while the second explores cosmetic products. The third chapter details the materials and methods, and the final chapter presents and discusses the results .

Part I
Bibliographic synthesis

CHAPTER I

Galactomannan

I.1 Generality

Most plants build big sugar molecules called polysaccharides using repeating small sugar parts locked together. Because these links hold firm, the whole structure stays strong and stable. One reason plants stand upright comes down to rigid layers made of cellulose, hemicellulose, pectin - materials that shape their outer framework. Inside cells, certain types like starch stock up energy when sunlight turns into food. Beyond support, such substances also help fend off threats from microbes or insects nearby. These natural plant materials do more than just support cell structure. They show strong health-related actions, like fighting oxidation or microbes, along with influencing immune responses - making them valuable across medicine, drugs, and food production **(Zhao *et al.,* 2023)..** Among them, one compound grabs attention: galactomannan. It belongs to a family of plant fibers called hemicellulose. Researchers are especially interested because it behaves in useful ways inside the body and performs well in practical uses.

I.2 Galactomannans

Found in legume seeds like guar, locust bean, and *Leucaena leucocephala*, galactomannans act as stored energy plus support structures within the seed's core **(García-Solís *et al.,* 2025).** A chain of mannose units linked by β-(1→4) bonds forms the main structure, while branches made of galactose attached via α-(1→6) linkages sprout off along it. Because of these side arms, the molecules mix well with water, turn solutions thick, and form stable gels when needed. Their ability to break down safely in nature, blend harmlessly with living tissue, and cause little to no toxicity opens doors across industries. Used without fuss in foods to adjust texture, they also show up in medicines as inactive helpers. Moisturizers rely on them just as much as delivery vehicles for drugs do - tiny particles or thin sheets built around this plant sugar behave predictably under stress. Even so, every role ties back to how the molecule interacts with liquid environments **(García-Solís et al., 2025).**

I.3 The molecular structure of galactomannans

Galactomannans have a straight chain made of D-mannose units joined by β-(1→4) bonds. Branching off this core, D-galactose units connect via α-(1→6) links at certain points **(Dias-Machado *et al.,* 2023).** Being built this way gives rise to a neutral molecule that attracts water easily. How it acts in liquid hinges on how far apart and how many galactose branches appear. Stiffness, water binding, and how molecules stick together shift

depending on where and how often those side groups show up. That alters thickness, whether it dissolves well, and if gels form. Along the main mannose line, plenty of –OH sites open doors for chemical tweaks. Adding sulfate or carboxymethyl groups reshapes performance - fine-tuning flow traits or fit within complex mixtures found in medicines, foods, lotions, or medical materials **(Dias-Machado et al., 2023).**

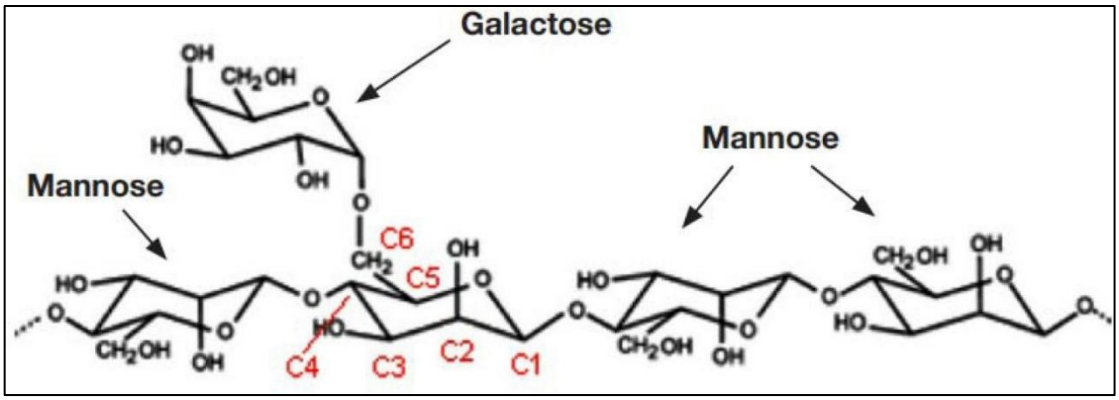


Figure 1. Schematic representation of galactomannan molecular structure chains

(Tedjani et al., 2025).

I.4 Structure and Function Relationship of Galactomannans in Various Plant Sources

Fenugreek gum, with lots of branches (G:M ≈ 1:1), mixes easily in cold water. On the flip side, locust bean gum has fewer branches (G:M ≈ 1:4), so it needs heat or help from other gels to dissolve fully **(Tedjani et al., 2025).** Guar gum sits in the middle - branching at about 1:2 - and balances how well it dissolves with how thick it gets. Because of this mix, it shows up often in foods, medicines, and factory-made products **(Tedjani et al., 2025).** Its shape gives decent flow control and fair solubility, much like tara gum. That quiet strength makes it interesting for uses people don't talk about much - like health boosters, plant-based wraps, or immune-supportive meals **(García-Solís et al., 2025).**

Table 1. Main galactomannan sources (Gabriela Ibieta et al., 2023 & Kontogiorgos, V. (2018).)

Gum name / source	Scientific name	Mannose: Galactose Ratio (Man:Gal)	Polysaccharide Yield (% dry seed weight)	Key Functional Attributes
Guar gum	Cyamopsis tetragonoloba	≈ 2:1	≈ 35–45%	High viscosity, strong cold-water thickener in food and industries.
Locust bean gum (LBG)	Ceratonia siliqua	≈ 4:1	≈ 30–40%	Moderate viscosity, strong gelling agent, synergizes with other gums.
Fenugreek gum	Trigonella foenum-graecum	≈ 1:1	≈ 25–30%	High solubility, good emulsifier and thickener for functional foods.
Tara gum	Caesalpinia spinosa	≈ 3:1	≈ 29–32%	Moderate viscosity, stabilizer and texture-modifier in dairy and bakery.
Leucaena leucocephala gum	*Leucaena leucocephala*	≈ 2.5–3.5:1	≈ 25–35%	Moderate viscosity and solubility; suitable for dietary supplements and functional foods.

I.5 Physicochemical Properties of Galactomannans

How much gets swapped out shapes things like how well it dissolves, its thickness, also how it mixes with other polymers or inactive ingredients. Their usefulness comes through clearly in medicines, skincare, even food - places that rely on them being plant-based gels, mix helpers, texture keepers **(Tedjani et al., 2025).**

I.5.1 Solubility

Water loves these sugar chains more when there's extra galactose tagging along - think lower mannan backbone traffic. Fenugreek and Chinese honeylocust versions slip right into room-temperature water without a fuss. Locust bean kinds? They resist, needing warmth and stirring to fully let go. Their sparse galactose placement leaves long stretches exposed, making them cling together instead of soaking up moisture **(Liu, Y et al., 2020)..**

I.5.2 Stability

Most galactomannans hold up well when heated or placed in different acid-base environments common in medicines and foods. Still, if left too long in very hot settings or highly acidic or basic solutions, they begin to break down slightly. This breakdown trims their size on a molecular level, making them less thick. Yet, when kept as a powder or solid, they stay unchanged over time**(Zhang et al., 2024).**

I.5.3 Viscosity

With rising concentration, longer chains, or more branches, galactomannan solutions thicken under still conditions yet thin when stirred - this flow behavior called shear-thinning depends heavily on structure. Guar-based types pack together easily, leading to higher thickness compared to others. Fenugreek-like versions carry extra galactose arms along their backbone, limiting how much they stretch out or tangle, which results in thinner mixes despite similar concentrations. These patterns emerged clearly in recent lab measurements tracking flow resistance across different forms **(Carvalho, R. et al., 2021).**

I.5.4 Interactions with Other Polysaccharides

When galactomannans meet other polysaccharides, hydrogen bonds take part - also hydrophobic patches stick together, while charged areas push or pull. These mixtures either team up well, split into layers, or build networks - the result hangs on molecular shape and how much mannose versus galactose is present. Strongest gels appear if little galactose sits along the chain; such high-mannose versions bond tightly with xanthan gum or some starch molecules. But when many side branches crowd the backbone, they get in the way - blocking close contact so cooperation fades fast **(Miyazawa et al., 2022).**

I.6 Biological Activities of Galactomannans

Some plant sugars show wide-ranging effects on health, opening doors for use in medicines, supplements, or skincare. Sourced mainly from seed-based plants like guar, fenugreek, or tara, these natural compounds break down safely in the body without harmful side effects. Research now points to their ability to influence immune responses, fight oxidative stress, feed beneficial gut microbes, while calming inflammation - findings supported by recent studies **(Tedjani et al., 2025; Tao et al., 2021; Naskar, A., & Kim, K. S. (2020)).**

I.6.1 Immunomodulatory Effects

Immune reactions get a nudge from galactomannans, waking up macrophages, dendritic cells, one kind of killer cell too. Breakdown pieces - say, those trimmed by enzymes - push white blood cells to swallow invaders faster, release more nitric oxide, pour out signaling proteins like IL-12 or IL-10, tilting immunity toward equilibrium between two key pathways **(Tao et al., 2021).** Inside living bodies, beta-type galactomannan pulled from guar gum revs gut defenses but avoids setting off alarm bells, marking it as gentle yet effective **(Wu, Y., et al., 2022).** Tiny carriers built on galactomannan backbones stretch how long vaccines stay active, keeping T-cells engaged longer than usual **(Mittal & Kaur, 2019).**

I.6.2 Antioxidant and Anti-inflammatory Activities

Tiny pieces of broken-down galactomannan grab free radicals fast, shown in lab tests using DPPH and ABTS methods - this helps protect cells from damage **(Tao et al., 2021).** When enzymes from Trichoderma reesei chop up these molecules, their ability to donate electrons jumps by 30–40%, likely because more OH and NH groups become exposed **(Tao et al., 2021)**. Inside simulated gut environments, these carbs quiet down angry signals like TNF-α and IL-6 while boosting calming ones such as IL-10, soothing irritated gut linings **(Wu, Y., et al., 2022).** In animal trials, milk treated with fermented galactomannan lowers signs of oxidation, pointing to hidden protective effects when eaten **(Carvalho, R., et al., 2021).**

I.6.3 Prebiotic and Gut Health Benefits

Though hard to break down in the stomach, galactomannans get processed by gut bacteria in the colon, producing short-chain fatty acids like acetate and butyrate. These compounds make the environment more acidic, slowing harmful microbes while strengthening the intestinal lining **(Tao et al., 2021).** Instead of vanishing unchanged, parts of these fibers turn into food for beneficial species - Bifidobacterium and Lactobacillus thrive when exposed to galactomannan oligosaccharides. As their numbers rise, so does overall microbial variety, leading to fewer cases of diarrhea in animals tested **(Naskar, A., et al., 2020).** When added at low levels - just 1 to 2 percent in feed - they help poultry grow faster and convert meals more efficiently. Because SCFAs assist in neutralizing toxins, birds consuming Gmos also show lower buildup of mycotoxins **(Faluan, S., et al., 2025).**

I.6.4 Anticancer and Antimicrobial Actions

Some tiny particles made from galactomannan carry chemo drugs like doxorubicin straight into tumor cells, killing them more efficiently than unbound medicine - especially in breast and colon cancers - needing less of the drug to work **(Tao et al., 2021).** Pulling out these compounds using just water from plant seeds stops harmful bacteria such as Staphylococcus aureus from sticking together and forming tough layers, even when present at moderate levels (MIC 15–30 mg/mL**) (Naskar, A., & Kim, K. S. (2020).** When processed with enzymes, galactomannans take on a different role entirely: they break apart fungal walls, weakening Candida species enough to stop their spread **(Wu, Y., et al., 2022).**

I.6.5 Wound Healing and Tissue Regeneration

Fibroblasts grow faster on galactomannan gels, while collagen builds up more quickly - wound healing speeds up in mice **(Naskar, A., & Kim, K. S. (2020).** Scaffolds made from electrospun *Leucaena leucocephala* galactomannan help skin cells move better because inflammation drops when NF-κB is quieted **(**Wu, Y., **et al., 2022)**

I.6.6 Metabolic and Antidiabetic Effects

Thickening the gut fluid helps soluble galactomannans delay sugar uptake, which then flattens blood spikes after meals **(Tedjani et al., 2025).** In lab animals with diabetes,

fenugreek's version - taken at 2–4 grams daily - tightened insulin response while cutting HbA1c levels by roughly a quarter **(Schalinske et al., 2024).**

I.7 Applications of Galactomannans

Found in more skincare mixes today, galactomannans come from plants and fit eco-friendly trends. These substances build texture, hold formulas steady, leave a light layer on skin, while softly improving feel - sometimes even helping fade age signs or brighten tone **(Flores García, et al., 2025)..** Because they hold lots of water, tend not to irritate skin, and mix well with many blending agents, these ingredients fit into countless beauty items - think facial moisturizers alongside wash-off treatments, SPF formulas rather than just leave-in conditioners. Though lightweight, their presence shows up across cleansers just as often as styling pastes, quietly supporting textures without drawing attention. What stands out is how gently they perform, whether sitting in a daily serum or overnight balm, working behind the scenes where stability matters most **(Guar gum cosmetic blogs, 2025; Reviews on galactomannan-based materials, 2025).**

I.7.1 Texturizing and Stabilizing Food-Grade-Like Polymers

Smoothness in facial products comes from gelling molecules shaping how thick or runny they feel. These ingredients build a slippery mesh when rubbed, letting go easily on skin yet holding firm inside the container. Gel structures respond to movement by thinning out just enough during application. What feels solid at rest turns fluid with motion. Stability stays intact until touch brings temporary change **(Flores García, et al., 2025).**Some makers swap out lab-made thickeners like carbomers or CMC using these ingredients instead. In products labeled clean beauty or eco-conscious, that shift matters more. Gel-forming molecules fit right in since they come from plants and break down naturally over time **(Industry blogs on guar-gum cosmetic applications, 2025; Comprehensive galactomannan-applications reviews, 2025).**

I.7.2 Moisturizing and Skin-Conditioning Agent

Moisturizers with galactomannan work by spreading a light layer over the outer skin. This shield holds in moisture, slowing down how fast water escapes through the surface. Skin feels smoother because it stays suppler longer. The film also supports

flexibility, helping maintain resilience. Less drying out means better protection overall **(Recent moisturizer-performance studies, 2025–2026; Cosmeceutical studies on galactomannan fractions, 2017–2024).** Starting off differently each time, these polymers team up well with classic moisture magnets like glycerin or hyaluronic acid, also working alongside softening agents in products meant for parched, reactive skin. Lightness stays intact, greasiness never shows up, yet deep-down wetness holds firm through the hours **(Hydration-performance studies, 2023; Artisan-formulator guides, 2025).**

I.7.3 Emulsion and Nano-Emulsion Stabilizer

Galactomannans contribute to emulsion stability in O/W and W/O systems by increasing the viscosity of the continuous phase and reducing droplet coalescence and phase separation **(Surfactant-free and modified-GM emulsion studies, 2021–2025; galactomannan-based review, 2025).** In advanced cosmetic systems, they are used in complex coacervate-based or nano-emulsion carriers to improve the shelf-life and controlled release of sensitive actives such as antioxidants, essential oils, and vitamins **(Minireviews on encapsulation using galactomannans, 2021; Cosmetic-emulsion studies, 2024).**

I.7.4 Active “Cosmeceutical” Ingredient

Some galactomannan parts, like those from Arenga pinnata, do more than affect texture - they act directly on skin cells. While most focus lands on flow behavior, these compounds engage living tissue in noticeable ways. Not just thickeners, they influence how skin functions at a basic level. From this source, specific segments trigger responses beyond physical effects. Though often overlooked, their role shifts when seen through a biological lens. These molecules participate, rather than simply sit within formulations

- Tyrosinase inhibition (lightening/whitening effect).
- Antioxidant activity via metal-ion chelation.
- When skin cells face UV light, compounds reduce MMP-1 alongside MMP-13 levels.

This shift slows visible aging signs over time. Responses inside damaged cells adjust when treated early. Lower enzyme activity supports firmer texture later on. Protection

emerges not from blocking rays but calming internal reactions. Effects show up clearly in lab tests using human tissue models.

These studies frame GM less as just a helper substance, more like a skincare-active with effects resembling treatments. One moment it sounds cosmetic, next it plays closer to medicine **(Liu, J. K. (2022).**

I.7.5 Hair-Conditioning and Styling Agent

With their ability to smooth strands and form protective layers, galactomannans now appear more often in shampoos, rinses, wraps, and hold gels. Because of how they stick to surfaces, charged versions of these molecules settle evenly on hair, cutting down tugging and split ends during brushing - yet still feeling airy and never tacky **(Wang et al., 2021).** From the seeds of Adenanthera pavonina comes a renewable option recently studied; it clings well to moist tissues and rarely causes discomfort, fitting well into formulas meant for delicate scalps or products that stay put all day **(Eid et al., 2025).**

I.7.6 Galactomannans in Sun Care, Makeup, and Cleansing Products

Galactomannans bring thickness to sun-care mixes, holding UV filters steady so they stay evenly spread across skin - smoother feel, easier glide comes along with that **(Flores García,, et al. 2025).**When found in makeup like foundation or tinted lotions, these compounds shape how the product flows, stopping colors from sinking and keeping blends intact over time**.** Found in face washes, micellar solutions, shower gels? They boost thickness even when surfactants are few, help particles stay suspended, soften harsh cleansing effects - gentle enough for delicate or dry complexions because they rarely cause reactions **(Flores García,, et al. 2025).**

Table 2. Main cosmetic applications of galactomannans (2021–2026)

Application area	Product type	Key functional role of galactomannans	Main cosmetic benefit
Face and body moisturizers	Creams, lotions, body butters	Moisturizing film-forming agent, humectant synergist	Improved hydration, reduced dryness, smoother skin
Facial gels and	Peel-off and	Gel-former,	Intensive, controlled

masks	wash-off masks	active-carrying matrix	release of actives, even film formation
Hair-care products	Shampoos, conditioners, styling serums	Hair-conditioning, anti-frizz, film-forming	Smoother, shinier hair; reduced breakage and static
Sun-care formulations	Sunscreen lotions, sprays	Emulsion stabilizer, texture modifier	Uniform UV-filter dispersion, pleasant non-greasy feel
Makeup and tinted products	Foundations, BB creams	Rheology modifier, pigment-suspension aid	Stable color, reduced settling and transfer
Cleansers and micellar waters	Facial cleansers, micellar solutions	Mild thickener, surfactant-stabilizing agent	Gentle, low-drying cleanse with improved foam and rinse-off

I.8.*Leucaena leucocephala*

I.8.1 Generality

Standing tall, this plant pushes upward fast - known as lead tree or simply leucaena. Up to 20 meters in good spots, though usually 3 to 15, it builds nitrogen as it races skyward **(CABI Compendium, 2023).** A member of the bean group called Fabaceae, it fits into fields and woods in more ways than one. Thanks to what it offers, farmers choose it for animal food, richer earth, holding dirt in place, also blending crops and trees. Feathers of pale green make up the foliage, branching into slender parts; meanwhile small creamy flowers bunch close, forming spheres. Roundness defines that bloom shape - which gave rise to the term leucocephala, translating to white head. After flowering comes flat fruit cases, filled with shiny dark seeds - one after another aiding its spread **(De Sousa Machado, M. T et al., 2020).** Rich in protein, leafy growth along with young pods feeds ruminants well; still trouble appears if mimosine, a rare amino acid, damages species unadapted to process it, making management key **(Padma Sharma , et al., 2022).** Now taking hold in many zones, *Leucaena leucocephala* grows dense, overtaking native flora especially where terrain has changed **(Chaer, G. M., et al., 2011) (Figure 02**)

Figure 2: *Leucaena leucocephala*(Trees and Shrubs Online. (2023).

Starting in Mexico and Central America, *Leucaena leucocephala* has spread into tropical and subtropical regions around the globe - reaching even sub-Saharan Africa **(Campbell, S., et al., 2019)..** Because of poor soil and low rainfall in parts of Africa, growers turn to this plant; it improves dirt health while offering feed after rain ends, along with fuel and timber, mostly within mixed farming systems involving trees **(CABI Compendium, 2023).** In countries such as Kenya, Tanzania, and Uganda, you see it often, since better land fertility links closely with higher livestock yields there **(Sharma, P.,et al., 2022).** Outside its native range, this species takes on dual functions - among them aiding recovery of damaged landscapes if managed thoughtfully. Still, it grows strong in spots such as southern Africa and scattered islands, overtaking local greenery. Along roads, beside rivers, on disturbed soil, its presence spreads fast. That change throws off natural balances, squeezing out original plants **(CABI Compendium, 2023).** For now, *Leucaena leucocephala* remains confined to trial areas in Algeria. While cultivation stays limited, research presses on in arid zones of the high plateau and sections of the northern steppe. One thing grabs attention: how little water it needs. That quality shows up alongside richer earth and quick growth, catching researchers' eyes. Thanks to these traits, ideas pop up - holding back sand drifts, softening gusty weather, giving empty plots a living cover **(Mohamed Rashid et al., 2021; AACAD, 2023)(Figure 3)**

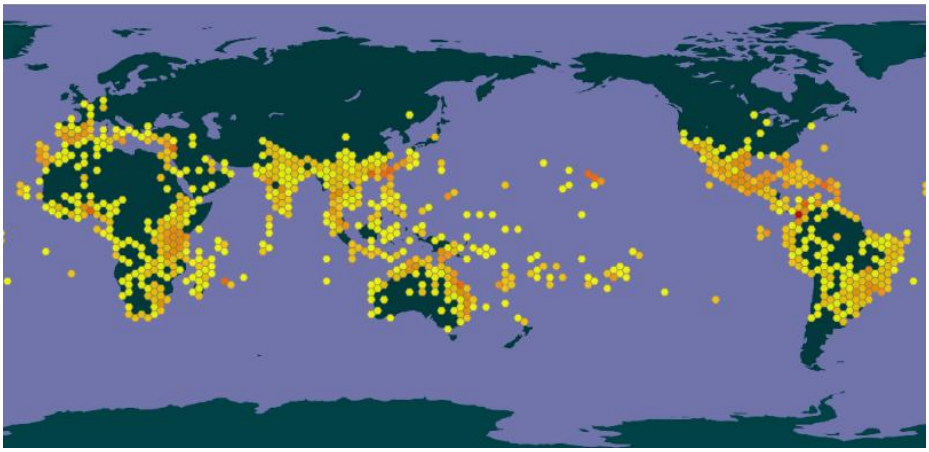


Figure 3. Global occurrence map of *Leucaena leucocephala***(GBIF.org (2024)**

Out pastures often host *Leucaena leucocephala,* its leaf matter packing a solid chunk of protein - around one-quarter dry weight - which works well for cattle once mimosine levels get managed. Since it pulls nitrogen straight from air, enriching tired soils becomes easier, sometimes adding hundreds of kilos per hectare annually while also holding loose earth in place, especially where land has weakened **(Casanova-Lugo, F.et al., 2014).**Across parts of sub-Saharan Africa, people weave this plant into mixed farms combining trees and animals, grabbing benefits beyond just animal food - firewood, sturdy poles - and seeing both livestock gains and worn-out plots come back slowly **(Padma Sharma et al., 2022).** Even under harsh skies such as Algeria's arid grasslands, trials are running because its long reach underground and fast climb above help block winds, slow desert creep, improve ground texture **(Mohamed Rashid et al., 2021; AACAD, 2023).** Nutrition hides not only in leaves but seedpods too - full of key amino acids like lysine and arginine - while trunks deliver burnable logs or building sticks, and lab tests reveal inner compounds made of flavonoids and phenols that resist decay-causing organisms **(Kekou, C., et al., 2020).**From seed sources, galactomannan appears - delivering texture plus gut benefits within food products **(Sagi, R., et al., 2025).** Even with spread concerns, certain types low in mimosine allow cautious use in farm landscapes **(Chaer, G. M., et al., 2011) (Table3)**

Table3. Main Uses

Use Category	Details	Reference
Fodder/Feed	High-protein leaves/pods for ruminants	Luo et al. (2022); Ham et al. (2025)
Soil Improvement	N-fixation, erosion control	Martínez-García et al. (2023)
Fuel/Timber	Firewood, poles in agroforestry	CABI Compendium (2023)
Desertification	Windbreaks, land restoration in Algeria	Negais et al. (2021)
Extracts	Antioxidants, galactomannan supplements	Kos et al. (2025)

I.8.2 Classification of *Leucaena leucocephala*

Flowering plants include *Leucaena leucocephala*, sorted into the Fabaceae family - also called Leguminosae - not far from the mimosoid group known as tribe Mimoseae, sitting inside Caesalpinioideae instead of standing apart as the old "Mimosoideae" subfamily once suggested **(Azani, N., et al., 2017).** Rooted in recent legume evolutionary maps, this arrangement reflects deeper genetic patterns pulled from large-scale phylogenomic work, anchoring Leucaena firmly as its own genus among the legumes (Legume Phylogeny Working Group, 2023).

Leucaena leucocephala stands recognized at the species level - a legitimate tree found across many warm regions, carrying subspecies like ssp. leucocephala and ssp. glabrata that show minor contrasts in surface hairiness and where they grow (**Tropical Forages, 2021;De Sousa Machado, M. T.,).** Placed within Plantae, it moves through layers: Spermatophyta or Angiosperms as division, then Eudicots or Magnoliopsida as class, followed by Fabales, Fabaceae, and finally Leucaena as genus, bearing the scientific tag *Leucaena leucocephala* (Lam.) de Wit, upheld globally (CABI Compendium, 2023; Plants of the World Online, 2024). Growing quickly while enriching soil with nitrogen, this plant fits naturally into tropical and subtropical environments around the planet. (Table 4)

Table 4. Scientific classification of *Leucaena leucocephala***(Azani, N., et al. (2023)**

Rank / Level	Scientific Name
Kingdom	Plantae
Division / Phylum	Tracheophyta / Angiosperms
Class	Magnoliopsida (Eudicots)
Order	Fabales
Family	Fabaceae (Leguminosae)
Subfamily	Caesalpinioideae (mimosoid clade)
Tribe	Mimoseae
Genus	*Leucaena*
Species	*Leucaena leucocephala (Lam.) de Wit*

I.8.3 galactomannan of *Leucaena leucocephala*

Found in the seeds of *Leucaena leucocephala*, galactomannan acts as a natural polysaccharide - part of the galactomannans family - and dissolves easily in water. This substance belongs among hydrocolloids that mix well with liquids. Its role spans across food and medicine, serving to thicken blends or keep mixtures stable. Scientists also explore its potential to deliver drugs effectively. Performance-wise, new findings suggest it matches up closely with widely-used alternatives like guar gum **(Naskar, A., & Kim, K. S. (2020))**.

Starting off, the galactomannan from *Leucaena leucocephala* features a chain of D-mannose units connected via β-(1→4) glycosidic bonds. Branching out from this core are D-galactose units, fastened by α-(1→6) linkages at the C6 site of those mannose pieces. Much like guar gum and related substances, its framework follows a familiar pattern - yet it stands apart due to variations in how much mannose there is compared to galactose. These shifts tweak how the molecule behaves functionally **(de Paula et al., 2021)**.

Some samples show a mannose to galactose ratio higher than 3:1, while in others it nears 1:1 - this shift comes from where the material is taken and its type, shaping how much the chains branch out, which then influences how well it dissolves and flows in water

(de Paula et al., 2021). Looking at methylated structures plus fragments made through acid breakdown shows roughly one quarter of the ends are free, with both sugars spaced fairly evenly across the chain, hinting that thickness in liquid stays quite consistent (**de Paula et al., 2021).**

Water grabs onto Leucaea leucocephala galactomannan easily, creating thick mixes without needing much of it - works best when the water isn't too acidic or hot. Outcomes from research point to its size being massive: bigger than 2,560,000 on the molecular scale

It flows well in water-based solutions because of its particular thickness - about 3.5 dl/g. This quality allows it to work alongside or replace certain standard hydrocolloids already on the market **(Naskar, A., & Kim, K. S. (2020)).**

With up to roughly 75% carbohydrates, the polymer includes small amounts of metals like Ca, Mg, K, and Na - these attach to hydroxyl groups found on the sugar structure. Because of their presence, interactions with metal surfaces might occur, possibly aiding complex salt creation **(de Paula et al., 2021)**.

Thickening water-based mixes? That's one way galactomannan from *Leucaea leucocephala* works - emulsifying too. Stability gets better in foods or medicines when oils mix with alginates or plant-derived fats. Scientists use it to build tiny carriers for medicine, like nanoparticles and gels that hold drugs inside. Eye treatments benefit because the material forms sponge-like networks letting medicine seep out slowly. Porosity helps control how fast substances enter the body. Controlled release finds support here, thanks to its water-loving structure **(Naskar, A., & Kim, K. S. (2020)).**

One way to change the polymer is through chemical tweaks, turning it into carboxymethyl-galactomannan - this version dissolves better, flows differently, holds on to metals like nickel and zinc more effectively. Even after these changes, it still keeps the natural biocompatibility that makes the original sugar chain useful. Because of this mix, scientists see chances to use it for pulling heavy metals out of environments or crafting smarter ways to deliver medicines **(Rodríguez, 2016)**.

Table 5. Comparison between *Leucaena leucocephala* Galactomannan and Guar Gum**(Naskar, A., & Kim, K. S. (2020).)**

Property	***Leucaena leucocephala* Galactomannan**	**Guar Gum (Conventional)**
Approximate M/G Ratio	≈ 1:1	≈ 1.6–2.0:1
Water Solubility	Water-soluble; forms viscous solutions	Water-soluble; high viscosity
Main Application	Thickener, drug carrier, nano-carrier	Food and industrial thickener
Approximate Molar Mass	>2,560,000Da	1000000

CHAPTER II

Cosmetic Products

II .1. Definition of Cosmetology

Cosmetology is a scientific discipline focused on developing and assessing products designed to clean, protect, and enhance the appearance of skin, hair, and nails. It draws on principles from chemistry, biology, and dermatology to ensure that everyday cosmetic items are both effective and safe (Baki & Alexander, 2022)

II .2. General definition of skin structure and function

The skin is the body's largest organ and functions as a sophisticated biological system made up of several layers and specialized structures that collectively provide protection, help regulate bodily functions, and mediate contact with the outside world. It is divided into three primary layers—the epidermis, dermis, and hypodermis—each containing unique cell types and serving specific physiological purposes **(Lai-Cheong & McGrath, 2021)**

II.3. Skin

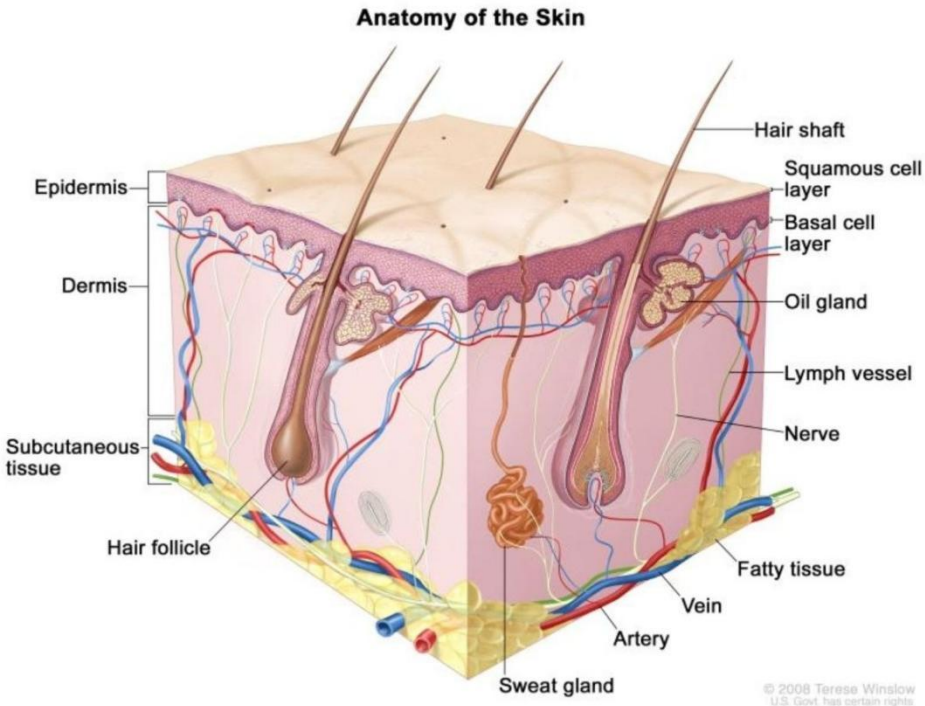


Figure 4: Anatomical Structure of Human Skin (NCBI Bookshelf, 2021)

II.3.1 Epidermis

The epidermis, the skin's outermost layer, is primarily made up of stratified squamous epithelial tissue. It acts as the body’s first line of defense, shielding it from environmental threats

like pathogens, chemicals, and ultraviolet radiation (Lim, 2021). This layer is structured into several sublayers, arranged from deepest to most superficial:

1. the stratum basale : which houses stem cells that drive ongoing cell regeneration
2. the stratum spinosum: which contributes to mechanical strength via interconnected keratinocytes
3. the stratum granulosum: playing a key role in producing keratin and forming a barrier against water loss
4. stratum corneum: composed of dead, keratin-filled cells that serve as a protective shield and help retain moisture **(Lefèvre-Utile et al., 2021).**

Main cells in the epidermis:

1. Keratinocytes: Generate keratin and contribute to the skin's protective outer layer.
2. Melanocytes: Synthesize melanin, which determines skin color and helps shield against ultraviolet radiation.
3. Langerhans cells: Play a role in the skin's immune response by detecting pathogens.
4. Merkel cells: Aid in sensing touch and relaying tactile information.

II.3.2 Dermis

Situated below the epidermis, the dermis is a layer of connective tissue that contains abundant extracellular matrix elements, including collagen and elastin. These components contribute to the skin's resilience and flexibility **(Lopez-Ojeda et al., 2022).**

Its main components include:

1. Collagen fibers: Offer resistance to stretching and provide structural strength.
2. Elastin fibers: Give skin the ability to stretch and recoil back to its original form.
3. Fibroblasts: Cells that generate collagen and elastin, supporting skin structure.
4. Blood vessels: Deliver essential nutrients and help control skin temperature.
5. Nerve endings: Detect sensory input such as pressure, pain, and temperature changes.
6. Hair follicles & glands: Contain sebaceous glands that produce oil and sweat glands involved in thermoregulation.

II .3.3 Hypodermis

The hypodermis, the deepest skin layer, consists primarily of adipose and loose connective tissue. It serves to attach the skin to deeper structures like muscles and bones **(Lopez-Ojeda et al., 2022).**

Key elements and their roles:

- Adipocytes (fat cells): responsible for energy storage and thermal insulation.
- Connective tissue: provides structural support and helps anchor the skin.
- Large blood vessels: play a role in regulating body temperature.

II.4. Functions of the Skin

The skin carries out several vital roles necessary for preserving the body's internal balance and providing protection. As a dynamic organ, it constantly engages with the external environment **(Lai-Cheong & McGrath, 2021).**

II.4.1. Protection Function

The skin's main role is to act as a protective barrier, shielding the body from physical, chemical, and biological threats—including ultraviolet (UV) radiation, microorganisms, and toxic substances. Central to this function are the stratum corneum and the lipids within the skin, which help minimize water loss and prevent the entry of harmful agents **(Lim, 2021; Lefèvre-Utile et al., 2021).**

II4.2. Thermoregulation

The skin plays a key role in regulating body temperature by managing processes like sweating and adjusting blood vessel dilation and constriction. These mechanisms support the maintenance of a consistent internal temperature, even when external conditions fluctuate **(Lopez-Ojeda et al., 2022).**

II.4.3. Sensory Function

The skin houses specialized nerve endings capable of sensing various stimuli, including touch, pressure, pain, and temperature. These sensory signals are relayed to the nervous system, enabling individuals to engage with their surroundings **(Lopez-Ojeda et al., 2022).**

II.4.4. Immune Defense

The skin functions as a dynamic immune organ, housing cells like Langerhans cells that play a role in detecting and combating microorganisms. Additionally, it fosters the skin microbiome, which helps defend against harmfulpathogens **(Lefèvre-Utile et al., 2021).**

II.4.5. Prevention of Water Loss

The skin serves as a protective barrier that helps minimize transepidermal water loss (TEWL), thereby supporting hydration and internal stability. This role is primarily linked to the epidermal barrier and the surrounding lipid matrix **(Lim, 2021).**

II.4.6. Vitamin D Synthesis

The skin is vital in producing vitamin D upon exposure to ultraviolet B (UVB) rays. This nutrient supports calcium regulation and contributes to maintaining healthy bones **(Sierra-Sánchez et al., 2021).**

II.4.7. Metabolic and Storage Functions

The skin is involved in various metabolic activities and acts as a storage site for lipids and water, primarily within the hypodermis, aiding in energy reserves **(Lopez-Ojeda et al., 2022).**

II.5. Skin pH

The degree of acidity or alkalinity at the skin's surface is referred to as skin pH. It is measured on a scale from 0 to 14, with values above 7 being alkaline, 7 being neutral, and values below 7 being acidic. Healthy human skin usually has a pH of 4.5 to 5.5, which is somewhat acidic and forms the "acid mantle" **(Lambers et al., 2021).**

• Role of Skin Ph

- Barrier function: Lipid structure and the integrity of the skin barrier are supported by the acidic pH.
- Hydration regulation: Preserves the action of enzymes involved in skin hydration and desquamation.
- Antimicrobial defense: It prevents the growth of harmful microbes while promoting healthy skin microbiota **(Lambers et al., 2021).**

II.6. Definition of a Cosmetic Product

A cosmetic product refers to any substance or mixture designed for application on external areas of the human body—including the skin, hair, nails, lips, and external genital organs—as well as teeth and the mucous membranes of the mouth. Its intended use includes cleansing, fragrance, altering appearance, protection, maintaining condition, or reducing body odor. Importantly, such products do not produce pharmacological or systemic effects **(StatPearls, 2023).**

II.7.Origine of a Cosmetic Product

The origin of a cosmetic product is determined by where its ingredients come from—whether they are derived from natural sources such as plants, animals, or minerals; synthesized chemically; or produced using biotechnological methods like microbial fermentation or enzymatic reactions. This distinction plays a key role in shaping the product's formulation, stability, and overall effectiveness **(Baki & Alexander, 2021).**

II.7.1. Natural Cosmetic Products

From nature come the bits that go into natural cosmetics - think plants, earth stuff, animals. Oils like those from argan or coconut trees slip right in. Extracts pulled straight from greenery show up often. Essential oils bring their usual scent. Clays dug from the ground play a role too. Waxes found in nature help hold things together. Little lab work touches these pieces along the way.

Some people like them because they mix well with the body, break down safely, yet can cause less redness - even so, what's inside shifts when surroundings change or how they're pulled out differs. Natural does not mean safe by default; keeping microbes at bay means adding preservatives anyway **(Nohynek et al., 2021).**

II.7.2. Non-Natural (Synthetic) Cosmetic Products

Synthetic cosmetic products consist of ingredients created through controlled chemical processes, as opposed to being extracted directly from natural sources. These man-made components are specifically designed to deliver reliable performance, along with enhanced purity and stability in cosmetic formulations **(Draelos & Thaman, 2022).**

II.8. Type of a cosmetics products

Cosmetics fall into a number of areas, including hair care products (shampoos), skin care products (moisturizers, toners, sunscreens), and other personal care formulas. Each variety is made to fulfill particular hygienic and aesthetic purposes while guaranteeing human body compatibility and safety**. (Kim et al., 2021)**

- Sunscreens
- Moisturizers
- Cleansers
- Toners
- Serums
- Body lotions
- Shampoos

Among the most popular skin care products are moisturizers, which are designed to keep skin hydrated, strengthen the skin's barrier, and improve the appearance of the skin in general.

They are vital to regular skincare regimens because they prevent transepidermal water loss and restore the physiological equilibrium of the skin. Moisturizers are a major area of interest in cosmetic research and formulation science because of their practical significance and extensive use**. (Draelos, 2022)**

II.9. Definition of Moisturizers

By lowering transepidermal water loss (TEWL), increasing the skin's capacity to retain moisture, and improving the integrity, elasticity, and general appearance of the skin barrier, moisturizers are topical formulations used in dermatology and cosmetology to maintain the water balance within the stratum corneum of the skin. **(Draelos, Z.D.,2022).**

Moisturizers are usually made up of a functional combination of occlusives, which create a protective layer that lowers water evaporation, humectants, which draw and bind water to the outer layers of the skin, and emollients, which fill the spaces between cells in the stratum corneum to improve skin smoothness and structural stability. As a result, moisturizers help prevent dryness and related dermatological disorders by not only hydrating the skin but also helping to maintain and repair the cutaneous barrier. **(Lodén, M.,2021)**

II.10. Moisturizing cream ingredients

• Humectants:

Humectants are hydrophilic compounds that draw and hold onto water molecules in the stratum corneum to improve suppleness and hydrate the skin. Glycerin, urea, hyaluronic acid, and sorbitol are typical examples. By increasing water content and promoting epidermal hydration mechanisms, these components are essential to contemporary moisturizers. (Draelos, 2022; StatPearls, 2023)

• Emollients:

Emollients restore lipid structure and fill the stratum corneum's intercellular gaps to make skin softer and smoother. They help to lessen roughness and rebuild the skin barrier.

Ceramides, fatty acids, cholesterol, and squalane are typical examples. Particularly for dry and sensitive skin, these lipids are crucial in formulations for barrier restoration. (Proksch et al., 2021; Draelos, 2022)

• Occlusives:

By creating a protective hydrophobic coating on the skin's surface, occlusive drugs dramatically lower transepidermal water loss (TEWL) and stop dehydration. Petrolatum, mineral oil, lanolin, and dimethicone are common occlusives. They work especially well in situations where the skin barrier is compromised and there is extreme dryness. (Rawlings, 2021; StatPearls, 2023)

• Excipients:

Or supporting formulation ingredients Creams include excipients that provide stability, safety, and usability in addition to active moisturizing ingredients. These include water as the primary solvent, pH adjusters to preserve skin compatibility, emulsifiers to stabilize oil-water systems, and preservatives to stop microbiological contamination. To enhance stability and sensory qualities, some formulations may also contain aroma compounds and antioxidants. (Benson et al., 2021; Draelos, 2022)

II.11.Types of moisturizing creams according to skin type:

❖ **Moisturizers for Dry Skin :**

Water-retaining creams tackle parched skin using ingredients that lock moisture in, fix the outer layer of skin. These products slow down water evaporation while adding back natural fats lost over time. Barrier support comes from substances that seal, attract, or smooth the surface - each playing a role in healing dryness **(StatPearls, 2023)**

❖ **Moisturizers for Oily Skin :**

Moisturizers for oily skin are lightweight, non-comedogenic formulations that hydrate the skin without boosting sebum production or clogging pores. They primarily contain humectants like glycerin and hyaluronic acid, with minimal lipids. These gel-based or oil-free moisturizers effectively maintain hydration while preserving the skin's natural sebaceous balance.**(Proksch et al., 2021; StatPearls, 2023)**

❖ **Moisturizers for Combination Skin :**

Moisturizers for combination skin are balanced formulations designed to care for both dry and oily areas of the face. They include moderate amounts of humectants and emollients to provide sufficient hydration without causing greasiness. Their semi-light texture ensures compatibility across various facial zones while supporting the skin’s barrier homeostasis.**(Draelos, 2022; Benson et al., 2021)**

❖ **Moisturizers for Sensitive Skin**

Moisturizers for sensitive skin are carefully formulated to reduce irritation and strengthen the skin barrier. They are typically fragrance-free and contain soothing agents like panthenol, allantoin, and ceramides. These ingredients work together to decrease inflammation, enhance epidermal repair, and restore barrier function in sensitive, reactive skin.(**Lodén, 2021; Draelos, 2022)**

❖ **Moisturizers for Normal Skin**

Moisturizers for normal skin are mild, balanced formulations aimed at maintaining natural skin hydration without disrupting its condition. They usually contain low to moderate levels of humectants and emollients, offering daily protection and hydration while supporting skin homeostasis **(StatPearls, 2023)**

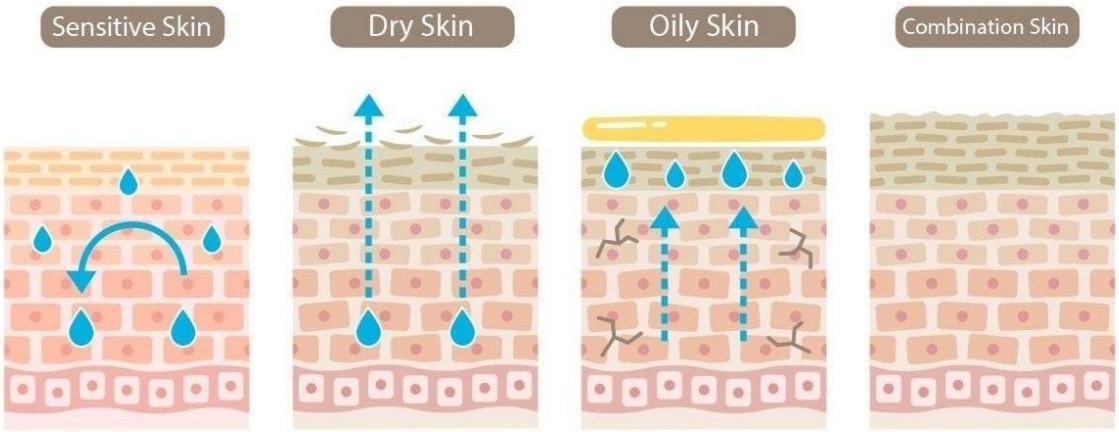


Figure 5. Types of Moisturizing Creams According to Skin Type (NCBI, 2021.)

II.12. Benefits of Moisturizing Creams

The restoration and preservation of the skin barrier function are the key dermatological advantages of moisturizing creams. Their primary functions include raising the stratum corneum's water content, lowering trans epidermal water loss (TEWL), and enhancing the general health and look of the skin.**(Draelos, 2022; StatPearls, 2023)**

- **Skin Hydration Improvement :**

Through humectants like glycerin and hyaluronic acid, which draw and hold moisture, moisturizers improve the stratum corneum's ability to bind water. As a result, the skin becomes more hydrated, more elastic, and less dry.**(Proksch et al., 2021)**

- **Skin Barrier Repair :**

By providing vital lipids including fatty acids, cholesterol, and ceramides, they aid in the repair of the epidermal lipid matrix. This strengthens the skin's defences against allergens and external irritants while also repairing damaged skin barriers.**(Lodén, 2021; Draelos, 2022)**

- **Reduction of trans epidermal water loss (TEWL) :**

By creating a protective layer on the skin's surface, occlusive substances like petrolatum and dimethicone dramatically lower trans epidermal water loss. In order to prevent dehydration and sustain long-term skin hydrated, this process is very crucial. **(StatPearls, 2023)**

❖ **Prevention of Dry Skin Disorders :**

By preserving skin hydration and barrier integrity, regular moisturizer use helps prevent xerosis (dry skin) and lessens the severity of dermatological diseases including eczema and atopic dermatitis.**(Draelos, 2022)**

❖ **Improvement of Skin Appearance :**

By filling in intercellular gaps and improving surface homogeneity, moisturizers improve the overall texture, softness, and smoothness of the skin. This results in skin that looks younger and healthier. **(Benson et al., 2021).**

Part II
Experimental section

CHAPTER III

Materials and methods

III. Materials and methods

This study aimed to prepare and evaluate a dermocosmetic moisturizing cream for topical application using galactomannan extracted from *Leucaena leucocephala* as a natural biopolymer. The work involved the extraction and purification of galactomannan to obtain a polysaccharide with suitable properties for dermocosmetic applications, followed by its incorporation into an oil-in-water emulsion. The formulated cream was subjected to physicochemical characterization, stability assessment, and microbiological evaluation. In addition to its moisturizing effect, the galactomannan was investigated for its potential role as a natural thickener, emulsifier, and humectant, offering a sustainable alternative to conventional synthetic cosmetic ingredients. Phytochemical analyses and total viable microbial count (TVMC) tests were performed in the Pedagogic Laboratory of the Faculty of Natural and Life Sciences at the University of El Oued, while pathogen identification analyses were conducted at the Laboratory for Analysis, Quality Control, and Compliance Assessment (Fatilab).

III. 1. Representation of the study area

Situated in southeastern Algeria, the Wilaya of El Oued lies within the northern reaches of the Sahara Desert and experiences a hyper-arid climate. Summer brings extreme heat, with temperatures often exceeding 48–50°C, while winter nights can be notably cold, occasionally approaching 0°C. Annual rainfall is very low, usually under 100 mm, contributing to chronic water scarcity and high rates of evapotranspiration. **(Bessaoud *et al.*, 2022) (Figure 6.).**

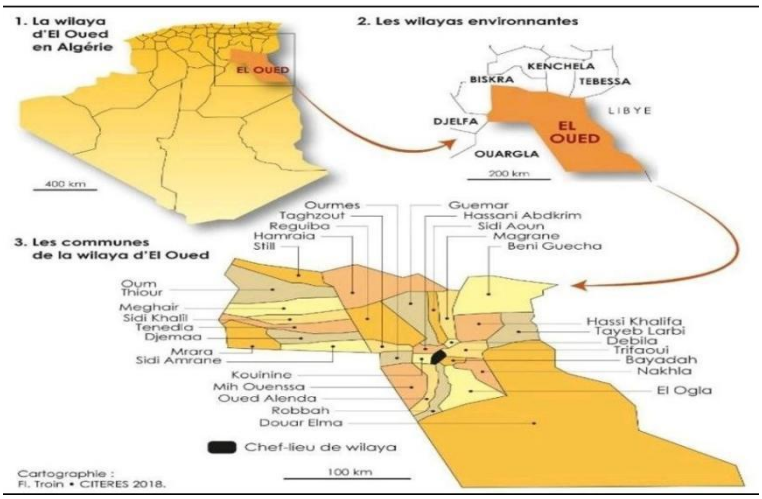


Figure 6. Geographic map of the El-Oued region **(Kadri & Chaouche 2018).**

III. 2. Plant harvesting

Leucaena leucocephala seeds were collected from the garden of the **Faculty of Science and Technology** at the University of **El Oued** (Algeria). **GPS coordinates: 33°22′56″ N and 6°51′52″ E**, during **November 2025**. The collection site was selected based on its controlled and relatively undisturbed environment to ensure the integrity and quality of the plant material. The choice of this species was motivated by the search for a promising source of natural polysaccharides. In particular, seeds were selected as the target plant part because they are known to be rich in polysaccharides, especially galactomannans.**Figure.7**

Figure.7*Leucaena leucocephala* (Original picture, 2026).

III. 3. Drying technique

The pods of *Leucaena leucocephala*, containing the seeds, were dried at room temperature (25–30°C) for two weeks on a kraft paper under ambient conditions until a constant dry mass was achieved. After complete drying, the seeds were manually separated from the pods. The collected seeds were then stored in a closed container and kept in a cool, dry, and dark place until further use. figure.8

Figure.8 The pods of *Leucaena leucocephala* (Original picture, 2026).

III.4 Extraction of the water-soluble Polysaccharides

The extraction protocol was adapted from the method described by **(Bia et al, 2025**), with slight modifications.

III.4.1. Step 01: Maceration

Using a series of aqueous extraction steps. Initially, **60 grams** of dried, ground seeds **(figure.9**) were dispersed in **450 mL** of distilled water and stirred at **65 °C** for **2 hours** at 500 rpm, leading to the formation of a viscous gel and the extraction process was repeated tree times using water 400 mL for the second and third extractions at 65 °C for 2 hours per extraction to ensure efficient recovery of polysaccharides. **figure.10**

Figure.9 Grinding seeds (Original picture, 2026).

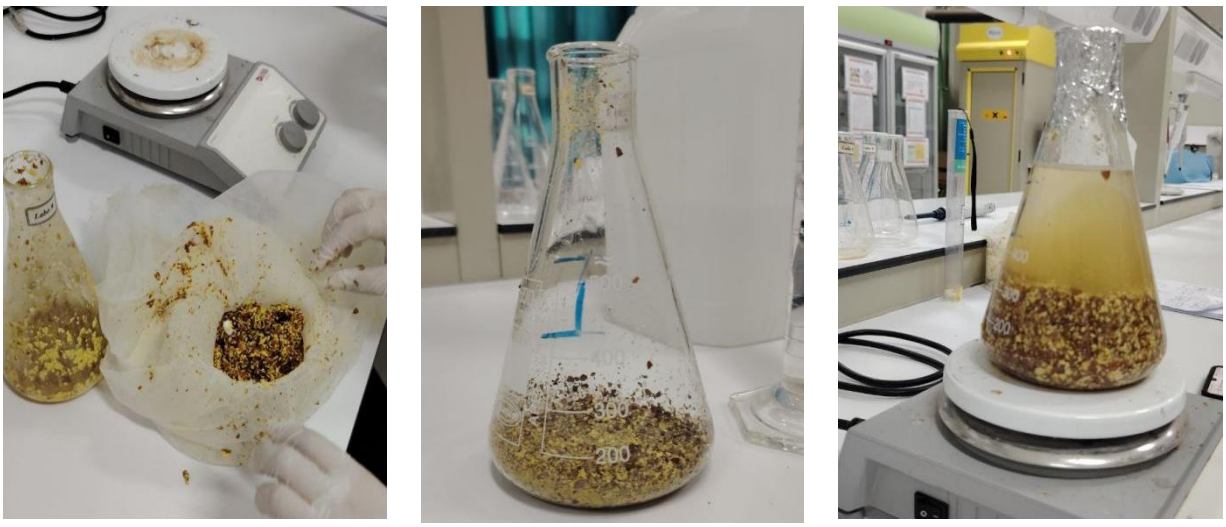


Figure.10 Maceration (Original picture, 2026).

III.4.2. Step 02: separation of gel

The mixture was cooled to 4°C and then centrifuged at 4000 rpm for 20 minutes to separate the gel. This process was repeated tow times. The supernatant from each tube we collected, Three volume of ethanol was added. The extract was placed in placed in a refrigerator overnight.

Figure.11 separation of gel (Original picture, 2026).

Figure.12 Precipitation of Polysaccharides (Original picture, 2026).

III.4.3. Step 03: Purification and Recovery of GSLL Polysaccharides

The precipitated material was washed initially with ethanol, followed by two successive washes with 30 mL of acetone to remove salts. After each washing step, the samples were subjected to centrifugation to ensure proper separation of the solid fraction from the washing solvents. The resulting material was then dried at 45 °C. Finally, the dried extract

was ground into a fine powder. A brown powder was obtained, referred to as GSLL, representing the galactomannan extracted from *Leucaena leucocephala* seeds.

- **Calculation of Extraction Yield**

The extraction yield was calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Mass of the extract (g)}}{\text{Mass of dry plant powder (g)}} \times 100$$

Figure.13 Purification of GSLL Polysaccharides **(Original picture, 2026).**

III.5 Composition of polysaccharides extract

The composition of total sugar, neutral sugar, protein, and polyphenols in the polysaccharide extract were measured using colorimetric methods.

For the solution preparation, **10 mg** of the polysaccharide extract was dissolved in **100 mL** of distilled water.

III.5.1 Determination of total sugars by the Dubois method

Principle

Total sugar content is measured using the colorimetric method described by Dubois et al. (1956). In acidic conditions, with concentrated sulfuric acid and heat, glycosidic bonds break down and the released monosaccharides undergo dehydration, producing furfural derivatives. These derivatives then react with phenol in a condensation reaction, resulting in a yellow-orange compound. The color intensity of this complex is quantified at 492 nm using spectrophotometry and correlates directly with the concentration of total sugars.

Materials and Reagents

A 5% (w/v) aqueous phenol solution was prepared using phenol from Sigma-Aldrich (PL037). Concentrated sulfuric acid (80%) was sourced from Honeywell (30743). A glucose standard (Sigma-Aldrich, G8270) was dissolved in water to achieve a concentration of 100 µg/mL and then serially diluted as needed. All procedures utilized distilled water. The sample tested were GSLL at a concentration of 0.01%., SHIMADZU UV-1800 spectrophotometer.

Procedure

- **To prepare a 5% phenol solution**, 1 g of phenol was added to 20 mL of distilled water and stirred until fully dissolved.
- **For the glucose stock solution (100 µg/mL),** exactly 10 mg of glucose was weighed and dissolved in 100 mL of distilled water **Table 06**

Table 6: Preparation of glucose standard calibration curve by the Dubois et al. method (1956)

Concentration (%)	Blanc	0.001	0.002	0.005	0.008	0.01
Eau distillée (µL)	200	180	160	100	40	0
Glc 100 µg/ml (µL)	0	20	40	100	160	200

- In glass test tubes, 200 µL of either the sample or standard solution was introduced and the tubes were placed in an ice bath. Next, 200 µL of a 5% aqueous phenol solution was added, and the contents were thoroughly mixed. Then, 1 mL of concentrated sulfuric acid (H_2SO_4, 80%) was slowly and carefully added. The mixture was heated at 90°C for 30 minutes, then allowed to cool for another 30 minutes at room temperature, protected from light. Absorbance was finally recorded at λ=490 nm.

III.5.2 Quantification of Neutral Sugars

Principle

The analysis of constitutive neutral sugars is performed using the colorimetric method outlined by Monsigny et al. (1988). It involves breaking down polysaccharide glycosidic bonds through hydrolysis with concentrated sulfuric acid under heated conditions. The resulting monosaccharides undergo dehydration to produce furfural derivatives, which then interact with resorcinol in an acidic environment. This reaction yields a brown-orange chromophore. Due to its high sensitivity, the method enables precise measurement of neutral sugars in the sample.

Materials and Reagents

Resorcinol (Sigma-Aldrich, product code 398047) was used to prepare an aqueous solution at a concentration of 0.6% (w/v). Honeywell (product code 30743) provided concentrated sulfuric acid (80%). A glucose standard (Sigma-Aldrich, G8270) was dissolved in water to achieve a concentration of 100 µg/mL, with further serial dilutions performed as needed. All procedures utilized distilled water. The sample under test was GSLL at a concentration of 0.01%. Measurements were carried out using a SHIMADZU UV-1800 spectrophotometer.

Procedure

- **To prepare a 0.6% resorcinol solution**, 6 mg of resorcinol was dissolved in 1 mL of Milli-Q water, resulting in a solution with a concentration of 0.6% (weight per volume). The solution was stored at 4°C and remained stable for up to four weeks under these conditions.
- **A glucose stock solution at 100 µg/mL** was prepared by dissolving 10 mg of glucose in 100 mL of distilled water. This initial solution served as the basis for subsequent serial dilutions needed to construct the calibration curve **(Table 07).**

Table 7. Glucose calibration curve preparation using the Monsigny et al. method

Concentration (%)	Blanc	0.001	0.002	0.005	0.008	0.01
Distilled water (µL)	200	180	160	100	40	0
Glc 100 µg/ml (µL)	0	20	40	100	160	200

▪ A volume of 200 µL of either the sample or standard solution was transferred into a test tube, then mixed with 200 µL of resorcinol solution and 1 mL of concentrated H_2SO_4. The contents were well mixed and heated in a dry water bath at 80°C for 30 minutes. Following incubation, the tubes were cooled to room temperature in the dark over a 30-minute period, after which absorbance was read at 450 nm using a spectrophotometer.

III.5. 3 Protein Quantification by the Bradford Method

Principle

The protein concentration is measured using the Bradford (1976) colorimetric method. This technique relies on the interaction between Coomassie Brilliant Blue G-250 dye and proteins in solution, forming a stable complex. The binding occurs mainly at specific amino acid residues—arginine, tryptophan, tyrosine, histidine, and phenylalanine—causing a visible shift in color that corresponds to the amount of protein present.

Materials and Reagents

Bradford reagent. Bovine albumin serum (BSA) (Sigma-Aldrich, A2153). Aqueous solution of BSA (100 µg/mL with necessary dilutions). Distilled water.

Sample to be tested (GSLL 0.01%). Vortex mixer. Spectrophotometer (SHIMADZU1800)..

Procedure

- To prepare the Bradford reagent, 100 mg of Coomassie Blue was dissolved in 50 mL of 95% ethanol. The solution was stirred continuously for two hours in the dark using a magnetic stirrer. Afterward, 100 mL of 85% orthophosphoric acid was added, and the volume was adjusted to 1 liter with distilled water.
- The finalsolution was filtered through filter paper and can be stored at 4°C for up to two weeks without significant degradation.
- A 0.01% BSA stock solution was prepared by dissolving 10 mg of Bovine Serum Albumin (BSA) in 100 mL of distilled water (see Table 08).

Table 8. Construction of BSA standard calibration curve by the Bradford protein assay

Concentration (%)	Blanc	0.0005	0.001	0.002	0.005	0.008	0.01
Distilled water (µL)	800	760	720	640	400	160	0
BSA 100 µg/ml (µL)	0	40	80	160	400	640	800

- 500 µL microliters of either the sample or standard solution were added to test tubes, followed by 500 µL of Bradford reagent. Each tube was vortexed for 5 seconds to achieve thorough mixing and then left to incubate at room temperature, protected from light, for 30 minutes. After incubation, absorbance was read at 595 nm with a spectrophotometer.

III.5.4 Quantification of Total Phenolic Compounds

Principle

The total phenolic content is measured using the colorimetric method described by Singleton et al. (1999). This method relies on the Folin–Ciocalteu reagent, composed of phosphomolybdate and sodium tungstate, which reacts with phenolic compounds to produce molybdenum and tungsten oxides. During the reaction, these metal ions are reduced, causing a rise in absorbance that is quantified at 765 nm. The resulting color intensity correlates directly with the concentration of phenolics in the sample.

Materials and Reagents

The Folin–Ciocalteu reagent (Sigma-Aldrich, F9252) was employed to measure total phenolic content. A 10% (w/v) aqueous solution of sodium carbonate (Sigma-Aldrich, S7795) was prepared for the analysis. Gallic acid (Sigma-Aldrich, G7384) served as the reference standard and was dissolved in water at a concentration of 100 µg/mL, with subsequent serial dilutions as needed. All procedures used distilled water. The sample tested were GSLL, both at a 0.01% concentration. Absorbance readings were taken using a SHIMADZU UV-1800 spectrophotometer.

Procedure

- Diluted Folin–Ciocalteu reagent (1:10) was prepared by mixing one part reagent with nine parts distilled water immediately before use.
- A 20% (w/v) sodium carbonate solution was made by dissolving 20 grams of sodium carbonate in 100 mL of distilled water.
- For the stock solution, 100 mg of gallic acid was dissolved in 100 mL of distilled water, which was subsequently used to generate the calibration curve **(Table 9).**

Table 9: Preparation of the gallic acid calibration curve using the Folin–Ciocalteu (Singleton) method

Concentration (%)	blanc	0.005	0.01	0.015	0.02	0.025	0,03	0.035
Distilled water (µL)	100	95	90	85	80	75	70	65
Gallic acid 100 µg/mL(µL)	0	5	10	15	20	25	30	35

- 100 µL of the sample was transferred into test tubes, followed by the addition of 500 µL of Folin–Ciocalteu reagent. The contents were thoroughly mixed using a vortex and left to stand for 3 minutes. Next, 2 mL of sodium carbonate solution was introduced, mixed briefly for 10 seconds, and then the mixture was kept in the dark at room temperature for one hour to allow the reaction to proceed. After incubation, absorbance readings were taken at 765 nm with a spectrophotometer.

III.6 Preparation of the cream

An oil-in-water (O/W) cream containing 1% *Leucaena leucocephala* mucilage as the active ingredient was formulated using an optimized emulsion preparation method. The proportions of the oil and aqueous phases, as well as the emulsifier, were determined after several optimization trials to achieve a cream with the most satisfactory organoleptic properties and physical stability **(Kumar et al., 2023; Al-Sayed & Ahmed, 2024).**

Vegetable oils, the emulsifier composed of Cutina and Emulgin B2, and other lipophilic components were dissolved in the oil phase (Phase B) and heated to 75°C. Meanwhile, the water-soluble components were dissolved in the aqueous phase (Phase A) and also heated to 75°C. Each phase was prepared in a separate container prior to emulsification, following modern pharmaceutical emulsion preparation protocols **(Singh et al., 2022; Patel & Sharma, 2023). Table 10 figure.14**

Table 10: Formulas and ingredients of elaborated cream **(MEKID & GHANES, 2021).**

Cream						
Ingredients	**Control**	**C1**	**C2**	**C3**	**C4**	**C5**
Aqueous phase A						
	40%	**60%**	**50%**	**40%**	**35%**	**40%**
Water	**36.3%**	**51.3%**	**41.3%**	**31.3%**	**26.3%**	**31.3%**
Glycerin	**3.7%**	**3.7%**	**3.7%**	**3.7%**	**3.7%**	**3.7%**
Mucilage	**/**	**5.0%**	**5.0%**	**5.0%**	**5.0%**	**5.0%**
Sodium Carbonate	**0.5%**	**0.5%**	**0.5%**	**0.5%**	**0.5%**	**0.5%**
Oily phase B						
	60%	**40%**	**50%**	**60%**	**65%**	**60%**
Emulsifier	**Emulgin B2**					**Cutina**
	20%	**20%**	**20%**	**20%**	**20%**	**20%**
Almond	**10%**	**6.5%**	**8.5%**	**10%**	**12.5%**	**10%**
Jojoba	**9.75%**	**3.25%**	**6.25%**	**9.75%**	**9.75%**	**9.75%**
Shea Butter	**9.75%**	**3.25%**	**6.25%**	**9.75%**	**9.75%**	**9.75%**
Lavender Oil	**10%**	**6.5%**	**8.5%**	**10%**	**12.5%**	**10%**
Additives C						
Vitamin E		**0.3%**	**0.3%**	**0.3%**	**0.3%**	**0.3%**

After the complete dissolution of the various ingredients, the aqueous phase was added portion-wise to the oil phase under continuous stirring at 75°C. Once the mixture was fully homogenized and a cream had formed, it was allowed to cool to room temperature.

Vitamin E was incorporated into the emulsion when the temperature dropped to 45°C, prior to transferring the final formulation into a suitable container.

Figure.14 Preparation Of The Cream (Original Picture, 2026).

III.7 Characterization of the cream

III.7.1 Organoleptic characterization

Organoleptic properties play distinct roles both in how a product is perceived prior to application and in its overall functional use **(Jacquemain, 1961)**. Based on the significance of these attributes, a sensory evaluation was conducted on all formulations listed in the menu, following the protocols established by **Mishraet al. (2014) and Sukamdi et al. (2024).**

This assessment, primarily relying on visual and tactile observation, allowed for the examination of several fundamental criteria: physical appearance (transparency or opacity), color, surface quality, texture (smooth or rough), and system homogeneity—particularly the presence or absence of phase separation or graininess. Additionally, the evaluation considered effects observed during application, such as drying behavior, sensations of tightness or stickiness, and the perception of shine.

III.7.2 Physico-chemical characteristics

III.7.2.1 Emulsion type

A drop of each cream mixed with a water-soluble red dye was examined under a microscope. If the dispersed globules appear colorless against a red background, the cream is an O/W type. The reverse condition occurs in the W/O type cream **(Mishra et al., 2014).**

III.7.2.2 Spreadability

It was determined by measuring the spreading diameter of a 1 g sample between two horizontal glass plates (10 cm × 20 cm) after one minute, under a standard weight of 200 g applied to the upper plate. Each formulation was tested in triplicate **((Sukamdi et al.,2024;; Gollavilli et al., 2020).**

$$S = \frac{m.l}{t}$$

- S: spreadability (g.cm.s-1)
- m: weight (200 g), t: time (60 s).
- l: surface area of cream spread on the slide

III.7.2.3 pH

To measure pH, 1 g of each formulation was mixed with 25 mL of distilled water, and the pH was measured using a pH meter. Prior to each measurement, the ph was calibrated with standard buffer solutions at pH levels of 4, 7, and 10. All tests were conducted in triplicate **((Sukamdi et al.,2024).**

III.7.2.4 The Sun Protection Factor (SPF)

the developed cream was assessed using the minimal erythemal dose (MED) resulting from UV radiation exposure. For SPF evaluation, 1% (w/v) of the cream was dissolved in a 40:60 ethanol-water mixture. A suitable dilution was then made, and the absorbance of the solution was measured between 290 and 320 nm at 5 nm increments (Poulose et al., 2020).

$$\mathbf{SPF = CF \sum_{290}^{320} EE(\lambda)I(\lambda)abs(\lambda)}$$

CF (Correction Factor): A constant value equal to **10**

EE: Erythemal effect spectrum (the relative effectiveness of UV radiation in inducing erythema at wavelength**λ**.)

I (λ): Solar intensity spectrum (the relative intensity of sunlight at wavelength **λ**).

Abs (λ): The experimental absorbance values of the cream sample measured at each specific wavelength.

The SPF is determined by the relationship between the erythemogenic effect (EE) and the radiation intensity at each wavelength (I) (Table 6), and is calculated using the previously mentioned equation.

Table 11. Correlation between the erythemogenic effect (EE) and the intensity of radiation at each wavelength **(Reis Mansur et al., 2016)**

λ(nm)	EE (λ) x I(λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

III.7.2.5 Stability tests

- Preliminary stability tests

These tests help to choose the ideal formula:

❖ **Stability following centrifugation**

The 24-hour samples were centrifuged at 3000 rpm for 30 minutes under room temperature conditions. Visual inspection, including assessment of appearance, uniformity, and organoleptic properties, was carried out through macroscopic analysis (Ostrosky et al., 2015).

❖ **Thermal stress stability**

The physical and chemical stability of the developed moisturizing creams under thermal stress was assessed using a modified approach derived from **Ostrosky et al. (2015).**

The cream samples were immersed in a temperature-regulated water bath and gradually heated from 40°C to 80°C. After reaching 80°C, the heating process was discontinued, and the samples were left to cool down on their own to ambient room temperature (25 ± 2°C).

To assess how the thermal treatment affected the formulations, organoleptic properties—such as appearance, color, and phase separation—along with pH levels, were measured at two stages: prior to heating and once the samples had completely cooled down.

❖ **Freeze/thaw cycle stability**

The samples underwent alternating conditions: first held at 4 ± 2 °C for 24 hours, then exposed to 45 ± 2 °C and 75% ± 5% relative humidity for another 24 hours, constituting one complete cycle **(Ostrosky et al., 2015)**. Prior to and following these initial stability assessments, evaluations included organoleptic properties, pH levels, and electrical conductivity **(Ostrosky et al., 2015).**

III.7.2.6 Viscosity

Principle

A rotational rheometer with plate–plate geometry is used to measure the viscosity of the cream. The technique evaluates the fluid's resistance to flow between two parallel plates, one stationary and the other rotating at a controlled shear rate. This allows characterization of the rheological behavior**. (Benaoun, 2017)**

Materials and Reagents

Rotational rheometer (plate–plate system), cream

Procedure

- The cream was formulated following the established standard protocol.

- A suitable amount of the product was placed onto the rheometer's measurement plate, which uses a parallel-plate configuration.

- The distance between the plates was carefully calibrated, and correct alignment was verified to ensure accurate measurements.

- The test temperature was stabilized at 21°C using the instrument's built-in temperature control.

- A shear rate sweep test was conducted, spanning from 0.1 to 100 s^{-1}.

- Apparent viscosity values were captured at each applied shear rate.

- Subsequently, the rheometer was programmed to step through decreasing rotational speeds, starting from 100 down to 2.5 s^{-1}, using its rotational control function.

- Viscosity readings were logged at every RPM setting along with their equivalent shear rates.

III.8. Microbiological quality testing of the cream

Objective

This study aimed to evaluate the microbiological quality of a water-in-oil (W/O)emulsion cosmetic cream by detecting and counting aerobic and mesophyll bacteria, as well as fungi, according to the methodology described by **Guiraud, 1998** and **Guleria, 2014.**

III.8.1. Total viable microbial count (TVMC)

III.8.1.1. Principle

The procedure involved inoculating specific growth media with cream samples and incubating them under controlled conditions. Any viable microorganisms present proliferate into visible colonies, quantified as Colony Forming Units (CFU). This quantitative assessment should be compared with the permissible microbiological limits established by the Official Journal of the People's Democratic Republic of Algeria, N°. 16 (2020), in order to assess its compliance and safety for topical use.

III.8.1.2. Materials and culture media

The microbiological evaluation of the formulated cream was carried out using standard laboratory materials and culture media. The main sample consisted of 1 g of the prepared cream. Microbiological culture media included Mueller Hinton agar for the detection of bacterial growth and Sabouraud Dextrose Agar for the detection of fungi. Physiological saline solutions were used for sample dilution, with a concentration of 0.45% (w/v) for fungal analyses and 0.9% (w/v) for bacterial analyses.

Sterile Petri dishes and sterile test tubes were used for culture handling and sample preparation. Sample distribution on agar surfaces was performed using sterile glass beads. Micropipettes (1000 µL) with sterile tips were used for accurate volume transfer. Homogenization of samples was carried out using a vortex mixer. Incubation of inoculated media was performed in anincubator at 25°C, 30°C, and 37°C. Sterilization of all glassware, media, and instruments was ensured using an autoclave prior to use.

III.8.1.3.Methods

❖ **Preparation of culture media**

Approximately 19 g of Mueller-Hinton agar and 30 g of Sabouraud agar powder were separately dissolved in 500 mL of distilled water with continuous stirring. Each medium was heated to boiling using a magnetic hot plate stirrer until complete dissolution **(Figure 15).** The prepared media were then sterilized by autoclaving at 121 °C for 20 min. After sterilization, the media were allowed to cool to approximately 45 °C then aseptically poured into sterile Petri dishes and left to solidify before use.

Figure 15. Preparation of culture media

❖ **Preparation of the Moisturizing Cream sample and serial dilutions**

First step: Preparing the sample and the original solutions

-A 2 g portion of cream was weighed and divided into two equal portions for microbiological analyses.

- For bacterial analysis, 1 g of the sample was aseptically transferred into 10 mL of sterile physiological saline solution (0.9% NaCl) to prepare the initial suspension.

- For fungal analysis, 1 g of the sample was similarly transferred into 10 mL of sterile physiological saline solution (0.45% NaCl).

- The suspensions were homogenized by vortex mixing for 2 min to ensure complete dispersion of the sample **(Guleria, 2014) (Figure 16).**

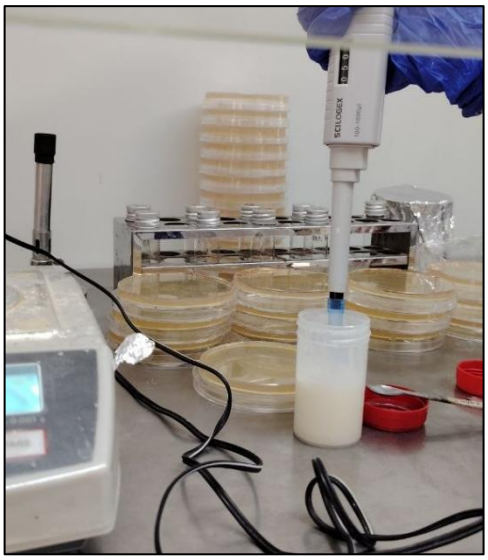


Figure 16. Preparing the sample and the original solution

Second step: Preparation of serial decimal dilutions

- One milliliter of the original suspension was transferred into a sterile tube containing 9 mL of diluent to obtain the first dilution (10^{-1}).
- The tube was mixed using a vortex for 15 s to obtain a homogeneous suspension.
- Next, 1 mL of the first dilution was transferred into another sterile tube containing 9 mL of diluent to obtain the second dilution (10^{-2}).
- The same procedure was repeated to prepare the third dilution(10^{-3}) (Figure 17).
- Sterile physiological saline solution (0.9% NaCl) was used for bacterial analysis, whereas a 0.45% NaCl solution was used for fungal analysis **(Guleria, 2014).**

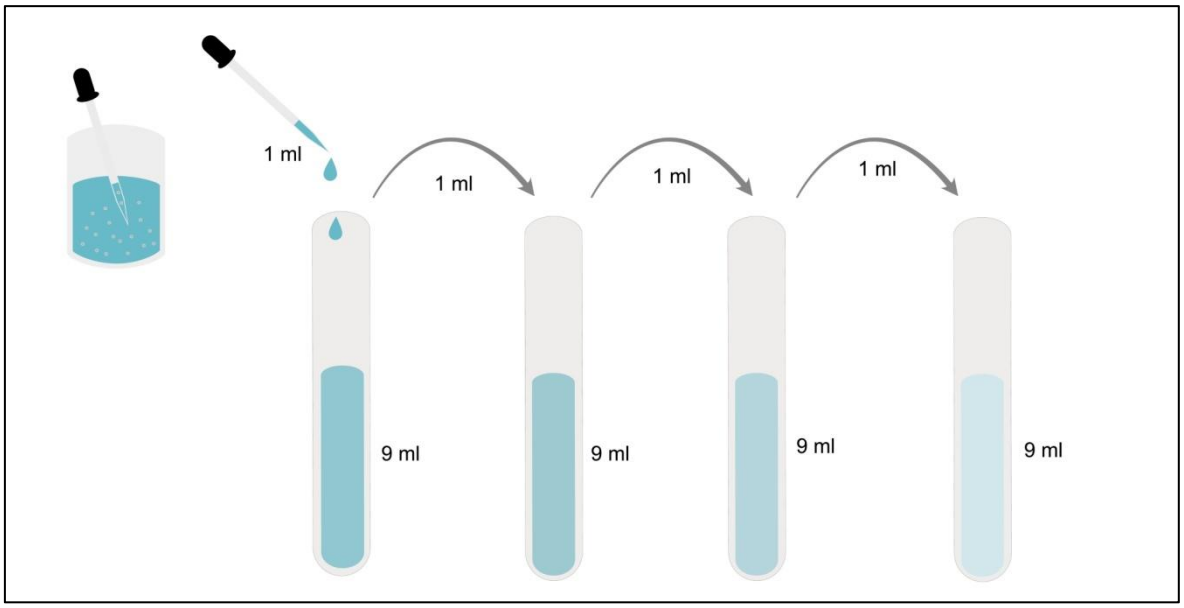


Figure17. Preparation of decimal dilutions of the cream.

Third step: Inoculation and Plating

-A volume of 100 µL was aseptically taken from each dilution.

-Each dilution was plated in triplicate onto sterile Petri dishes containing Mueller-Hinton agar for bacterial counts and Sabouraud dextrose agar for yeast and mold counts.

-The inoculum was spread evenly over the agar surface using sterile glass beads. The plates were gently rotated to ensure uniform distribution, after which the beads were removed aseptically **(Figure18).**

-The plates were incubated in an inverted position. Bacterial cultures were incubated at 30–37 °C for 24–48 h, while yeasts and molds were incubated at 28 °C for up to 7 days.

-Total bacterial and fungal counts were determined using the plate count method **(Spencer and Spencer, 2001; Da Silva *et al.,* 2018).**

- Each experiment was conducted in triplicate at four sampling times (24 h, 7 days, 16 days, and 21 days) to ensure the accuracy and reproducibility of the results.

-All procedures were performed under sterile conditions (under a bunsen burner or in a sterile chamber).

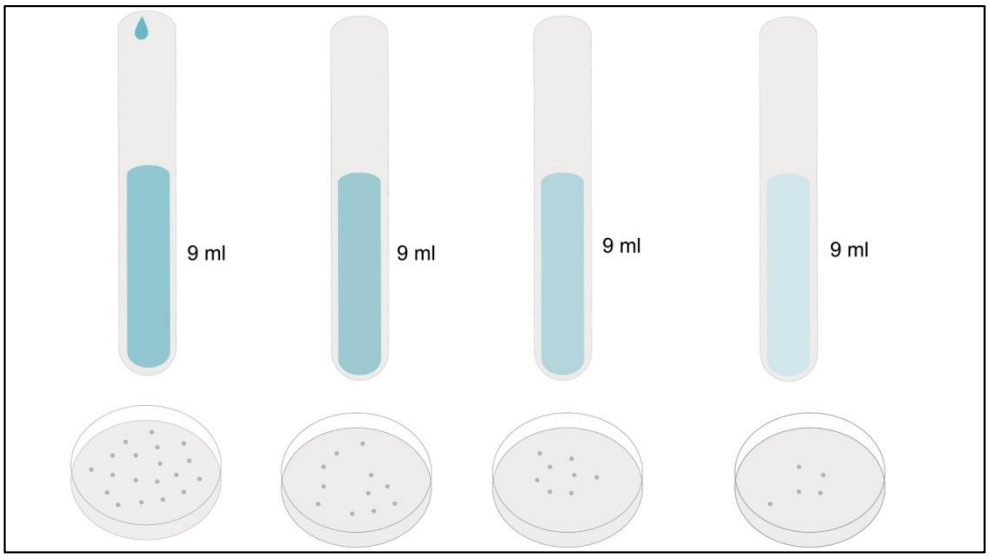


Figure 18. Pouring and planting method

III.8.2. Detection of the pathogen bacteria

III.8.2.1. Principle

Pathogenic bacteria detection and enumeration were based on enrichment, serial dilution, and selective culture techniques to determine the presence or absence of pathogenic microorganisms as well as the total microbial load in cream samples after incubation on appropriate culture media.

III.8.2.2. Materials and reagents

- **Culture media:** Nutrient Agar (NA), Blood Agar (BA), Nutrient Agar Slants, Eugon LT 100 Broth, MacConkey Agar (MAC), Eosin Methylene Blue Agar (EMB), Cetrimide Agar, Pseudomonas Agar P, and Baird–Parker Agar (BPA).
- **Reagents and diluents:** Sterile physiological saline solution (0.9% NaCl), Gram staining reagents (crystal violet, Gram's iodine, decolorizer, and safranin), oxidase reagent, 3% hydrogen peroxide (catalase reagent), and rabbit plasma (coagulase reagent).
- **Samples:** 1 g of cream sample used for pathogen detection and enrichment procedures.
- **Equipment:** Sterile Petri dishes, sterile test tubes, sterile inoculating loops, sterile cotton swabs, micropipettes with sterile tips, vortex mixer, UV lamp, light microscope, autoclave, incubators (32.5 ± 2.5 °C and 37 °C), analytical balance, and refrigerator (4 °C).

III.8.2.3. Method

1. Isolation and purification of pathogenic bacteria

Under aseptic conditions, each sterile swab was streaked onto two solid culture media, namely nutrient agar and blood agar. The inoculated plates were incubated at 37 °C for 24 h and subsequently examined for the presence of bacterial growth **(Saleem *et al.,* 2018; Agi *et al.,* 2023; Ebah *et al.,* 2024).** Distinct colonies were then subcultured onto nutrient agar to obtain pure isolates. The purified cultures were maintained on nutrient agar slants and stored under refrigeration for subsequent analyses **(Monica, 2006).**

2. Identification of pathogenic bacteria

Isolated bacterial strains were identified according to their morphological features and biochemical profiles using standard biochemical tests **(Bergey and Holt, 2000; Saleem *et al.,* 2020; Urooj *et al.,* 2022; Aernan *et al.,* 2023).** The identification of the specified pathogenic microorganisms were carried out according to ISO 18415:2017, with specific procedures for *Escherichia coli* (ISO 21150), *Pseudomonas aeruginosa* (ISO 22717), *Coagulase-positive staphylococci* (ISO 22718).

❖ **Identification of *Escherichia coli***

The detection of *Escherichia coli* was performed according to the principles of ISO 21150:2015. Briefly, 1 g of the sample was enriched in 9 mL of Eugon LT 100 broth and incubated at 32.5 ± 2.5 °C for 20–72 h. Following enrichment, a loopful of the culture was streaked onto MacConkey agar and incubated at 32.5 ± 2 °C for 24–48 h. Presumptive *E. coli* colonies appeared as brick-red colonies with bile precipitation. Suspected colonies were subjected to Gram staining, and Gram-negative bacilli were further confirmed by subculturing on Eosin Methylene Blue (EMB) agar. After incubation at 32.5 ± 2 °C for 24–48 h, *E. coli* colonies were identified by their characteristic metallic green sheen (Condalab, 2020).

❖ **Identification of *Pseudomonas aeruginosa***

The detection of *Pseudomonas aeruginosa* was carried out according to the principles of ISO 22717:2015. A 1 g of the sample was enriched in 9 mL of Eugon LT 100 broth and incubated at 32.5 ± 2.5 °C for 20–72 h. Following enrichment, a loopful of the culture was streaked onto cetrimide agar and incubated at 32.5 ± 2.5 °C for 24–48 h(Condalab, 2020).

Presumptive *P. aeruginosa* colonies were identified by the production of greenish-yellow fluorescent pigments under UV light. Well-isolated colonies were subjected to Gram staining and the oxidase test. For confirmation, suspected colonies were subcultured onto Pseudomonas Agar P and incubated at 32.5 ± 2.5 °C for 24–72 h.

The presence of *P. aeruginosa* was confirmed by the development of colonies surrounded by a greenish- blue pigment (pyocyanin) or a red-brown pigment (pyorubin)(Condalab, 2020).

❖ **Identification of *Coagulase-positive staphylococci***

The detection of *Coagulase-positive staphylococci* was performed following the guidelines of ISO 22718:2015. Briefly, 1 g of the sample was inoculated into 9 mL of Eugon LT 100 broth and incubated at 32.5 ± 2 °C for 20–72 h. Following enrichment, the culture was streaked onto Baird–Parker agar and incubated at 32.5 ± 2 °C for 24–48 h.

Presumptive *Coagulase-positive staphylococci* colonies were identified by their characteristic shiny black appearance surrounded by a clear halo. Suspected colonies were further examined by Gram staining and subjected to catalase and coagulase tests. The identification of *Coagulase-positive staphylococci* was confirmed by the presence of Gram-positive cocci arranged in clusters together with positive catalase and coagulase reactions (Condalab, 2020).

III.8.3. Interpretation criteria

- **Total viable microbial count of bacteria**

The microbiological results were interpreted according to the criteria established by the Official Journal of the People's Democratic Republic of Algeria, No. 16 (2020). In this regulation, m represents the microbial limit below which the product quality is considered satisfactory **($m \leq 10^3$)**, whereas M represents the limit above which the product quality is considered unsatisfactory **($M \leq 2 \times 10^3$).**

- **Total viable microbial count of molds and yeasts**

If the analytical result is less than or equal to m **($m=M \leq 10^2$),** the result is considered satisfactory.

If the analytical result exceeds **m**, the result is considered unsatisfactory.

Where:

m (= M): the number of yeasts and molds present in one gram or one milliliter of the analyzed product, representing the maximum acceptable limit. Any value exceeding this limit is considered microbiologically unsatisfactory.

Bacterial and fungal populations were quantified and expressed as colony-forming units per gram (CFU/g). Enumeration was based on dilution plates yielding **30–300** colonies, in accordance with standard microbiological counting practices. The CFU/g values were calculated using the following equation:

$$N = \frac{\Sigma C}{(n_1 + 0.1 n_2) \times d}$$

ΣC: is the sum of colonies counted on selected plates.

n_1 and n_2: are the number of plates at two successive dilutions.

d : is the lowest selected dilution

- **Interpretation of the results for the detection of pathogenic microorganisms**

-The result is considered satisfactory when pathogenic microorganisms are absent from the sample.

-The result is considered unsatisfactory when the presence of pathogenic microorganisms is detected in the sample.

III.9. Biological Activities

III.9.1. Evaluation of antioxidant activities by DPPH free radical scavenging

Principle

The DPPH• scavenging assay is based on the reduction of the stable violet DPPH• radical into a pale yellow non-radical form 2,2-diphényl-1- picrylhydrazyl by accepting a hydrogen atom or an electron from an antioxidant compound **(Ahmad *et al.,* 2021; Baliyan *et al.,* 2022).** This chemical reaction causes a distinct discoloration from violet to yellow, which is directly monitored via a spectrophotometer at a maximum absorbance of 517 nm **(Ali *et al.,* 2023).** The decrease in absorbance is proportional to the radical scavenging capacity of the sample, providing a rapid measure of its antioxidant efficiency **(Saleh *et al.,* 2024).**

Materials and Reagents

- The chemical reagents and materials required for the DPPH• radical scavenging assay include **(Ahmad et al., 2021; Baliyan *et al.,* 2022):**

- **DPPH• Reagent:** 2,2-diphenyl-1-picrylhydrazyl free radical (Sigma-Aldrich or equivalent).
- **Solvents:** Analytical grade Methanol or Ethanol (≥ 99.5) used for DPPH• dissolution and sample extraction.
- **Reference Standards (Positive Controls):** Ascorbic acid (Vitamin C), Gallic acid, or BHT (Butylated hydroxytoluene) **(Ali *et al.,* 2023).**
- **Apparatus:** UV-Vis Spectrophotometer (or microplate reader), vortex mixer, analytical balance, and dark/amber glass vials to protect the light-sensitive DPPH• solution **(Saleh *et al.,* 2024).**

Procedure

The DPPH• radical scavenging activity was determined according to the standard method with slight modifications **(Ahmad et al., 2021; Baliyan et al., 2022)**:

1.Preparation of DPPH solution : 9.858 mg of DPPH is dissolved in 100 mL of ethanol with continuous stirring. The solution is protected from light by covering it with aluminum foil and used immediately.

2.Preparation of ascorbic acid solution : 10 mg of ascorbic acid is dissolved in 100 mL of distilled water and used as a positive control

Table 12 : Serial dilution

Concentartion	0.1	0.08	0.04	0.02	0.008	0.004
distilled water(µL)	0	200	400	800	900	900
Ascorbic acid µg/mL(µL)	1000	800	600	200	from 0.08 tube 100 µL	from 0.04 tube 100µL

Preparation of sample solution : 10 mg of cream is dissolved in 10 mL of distilled water

Table 13 : Serial dilution

Concentartion	1	0. 8	0. 4	0.1	0.05	0.01
distilled water(µL)	0	200	600	900	950	990
DPPH	1000	1000	1000	1000	1000	1000

In each tube, the reaction mixture comprised 1 mL of sample solution and 1 mL of DPPH• reagent. This mixture was incubated for 30 min in the absence of light, after which its absorbance was recorded at 517 nm against the blank.

III.9.2 Evaluation of antibacterial activity

Principle

This technique involves placing a sterile disc, soaked with a cream, onto an agar surface that has been evenly inoculated with bacteria. As the bacterial culture starts to develop, the extract may prevent growth in the area surrounding the disc. The clear zone that forms—where bacterial growth is inhibited—is measured to assess the diameter of inhibition, which reflects the cream's antibacterial activity. The disc diffusion method **(Ghoul et al., 2024)** was used to evaluate the cream's antibacterial effectiveness.

Materials and reagents

Filter paper (Wattman's), Muller Hinton agar, petri dishes, Pasteur pipette, ecouvillon, Escherichia coli 25322, Staphylococcus aureus 25923, Gentamicin (GMN).

Procedure

The cream's antibacterial properties were assessed using the agar diffusion technique, based on the method outlined by Ojagh et al. (2010), with slight modification . The evaluation included four pathogenic bacterial strains: Escherichia coli (ATCC 25922), and Staphylococcus aureus (ATCC 25923). These bacterial cultures were stored at 4 °C on nutrient agar and routinely subcultured every 24 hours before testing.

a. Preparing Mueller-Hinton Agar (MHA)

- Weighing 38 grams of commercially available Mueller-Hinton agar powder per liter of distilled water..
- Heat the mixture with constant stirring to fully dissolve the powder, continuing until the solution is clear.
- Sterilize the solution by autoclaving at 121°C for 15 minutes under a pressure of 15 psi.
- After sterilization, let the medium cool to a temperature between 45 and 50°C.
- Under aseptic conditions, dispense about 20 to 25 mL of the warm medium into each sterile Petri dish.
- preparing nutrient agar medium

b. To prepare the medium, 28 grams of agar powder were weighed out for every liter of distilled water. The mixture was heated with constant stirring until the agar completely dissolved, usually through gentle boiling for a few minutes. It was then sterilized in an autoclave at 121°C and 15 psi for 15 minutes. After sterilization, the solution was cooled to between 45°C and 50°C. Under sterile conditions, 20 to 25 mL of the warm agar were poured into clean Petri dishes. The plates were left at room temperature to solidify and then stored inverted at 4°C until needed..

c. The bacterial strains were first activated by incubation in agar medium, followed by growth in agar broth at 37 °C for 24 hours. Afterward, 100 µL of each bacterial suspension—adjusted to a concentration of approximately 10^8 CFU/mL—was uniformly spread onto Mueller-Hinton agar plates. Sterile paper discs (6 mm in diameter) were soaked with 50 µL of cream solution at varying concentrations and placed onto the inoculated agar surface. Additional discs were similarly treated with 50 µL of gentamicin and saline solution and positioned on the other plates. All plates were then incubated at 37 °C for 24 hours. Antimicrobial activity was evaluated by measuring the diameter of the clear zones of inhibition surrounding the discs **(Mahcene, 2021).**

CHAPTER IV

Results and discussion

IV. Results and discussion

This chapter presents the physicochemical characterization and application of the extracted polysaccharides, as well as the microbiological quality control tests performed to evaluate the safety and suitability of the formulated product.

IV.1 Extraction yield

The polysaccharides was extracted from the *Leucaena leucocephala* seeds which were harvested from El Oued province (Septentrional Algerian Sahara) by hot water maceration in (65°C) for two hours. This extraction facilitated the production of a gelatinous extract, with an extraction yield ratio of about (11.66%), as shown in **Table (14)**

Figure 19. Image of the seeds used and the extracted gum powder.

The yield of water-soluble polysaccharides extracted from *Leucaena leucocephala* seeds (11.66%) was lower than that reported for Cyamopsis tetragonoloba (guar gum) seeds, which reached 32.40% under optimized aqueous extraction conditions **(García- Solís, P., et al. (2025).).** However, this value was notably higher than the yield from Indigofera trita seeds, which accounted for only 3.34% of total plant mass **(Tedjani, A., et al., 2025).** These observed fluctuations in gum and polysaccharide yields among the Fabaceae family are primarily governed by genetic traits, seed coat thickness, and structural tissue configurations. Additionally, parameters such as the mechanical pre-treatment of the seeds, water-to-raw-material ratios, and thermal processing during extraction play a critical role in determining the final recovery percentage.

IV.2 Chemical composition of the crude polysaccharides extract

Chemical analysis of the polysaccharide fraction extracted from *Leucaena leucocephala* seeds showed it to be predominantly composed of carbohydrates, indicating successful isolation of a galactomannan polymer. Using the phenol-sulfuric acid method, the total sugar content was measured at 87.52 ± 0 %, a notably high purity level that exceeds values previously reported for other species within the genus, such as Leucaena diversifolia, where carbohydrate content remained below 79,5% (**Shirajuddin, S., et al., 2015**)Of particular importance, the neutral sugar content—assessed through the resorcinol-sulfuric acid assay—was found to be 62.98 ± 3.62%. This substantial proportion supports the predominance of mannose and galactose in the polymer's main chain, a feature consistent with the structural profile typical of high-grade seed gums from the Fabaceae family **(Dholakia et al., 2022).**

In contrast, protein levels measured using the sensitive Bradford assay were found to be 15 ± 0.43%. Although this level suggests a semi-purified botanical extract, it still offers considerable benefits for specific cosmetic and pharmaceutical applications. In emulsion systems like cosmetic creams, these naturally co-extracted proteins work in synergy with the galactomannan structure, markedly improving emulsification efficiency, droplet stability, and the formulation's overall structural robustness Although the mechanisms can vary from steric stabilization to complex biopolymer interactions as reviewed by **Ai et al. (2021),** the co-presence of both macromolecules effectively prevents droplet coalescence and enhances cream texturization."

The total polyphenol content in the extract was relatively low, measured at 4.74 ± 0.27%, which aligns with typical findings for seed endosperm. Such reduced levels are expected when compared to leaf tissues, where polyphenol concentrations often surpass 5% due to photosynthetic activity and environmental stress responses **(Wang et al., 2017).** The limited presence of phenolic compounds is advantageous, as it reflects high extract quality by promoting color stability and minimizing oxidative degradation during storage. As noted by **(Dholakia et al., 2022). Table (14)**

Table 14. Chemical composition of the water-soluble polysaccharides extracted from *Leucaena leucocephala.*

Yield (%)	Total sugar (%)	Neutral sugar (%)	Proteins (%)	Polyphenol (%)
11.66	87.52 ± 0	62.98 ± 3.62	15 ± 0.43	4.74 ± 0.27

IV.3 Preparation of the cream

Developing a cream using *Leucaena leucocephala* gum involved multiple steps to fine-tune the proportions of phases and the concentration of emulsifiers. Early trials faced issues like phase separation and insufficient viscosity. These problems were overcome by adjusting the emulsification method and keeping both phases at a constant temperature of 75°C, ultimately yielding a stable oil-in-water (O/W) formulation. The use of Emulgin B2 proved highly effective as an emulsifier, successfully incorporating 5% of the gum into the water phase. This produced a cream with a smooth consistency and favorable sensory characteristics, aligning with results reported by **Sharma et al., (2025).**

Table 15: Composition of the optimized O/W cream

Cream		
Ingredients	**Control**	**C3**
Aqueous phase A		
	40%	**40%**
Water	**36.3%**	**31.3%**
Glycerin	**3.7%**	**3.7%**
Mucilage	/	**5.0%**
Sodium Carbonate	**0.5%**	**0.5%**
Oily phase B		
	60%	**60%**
Emulsifier	**20%**	**20%**
Almond	**10%**	**10%**
Jojoba	**9.75%**	**9.75%**
Shea Butter	**9.75%**	**9.75%**
Lavender Oil	**10%**	**10%**
Additives C		
Vitamin E	**0.3%**	**0.3%**

This well-developed cream formulation offers a stable and consistent base suitable for additional testing, such as long-term stability studies, pH measurements, and evaluation of microbiological quality.**Figure 20**

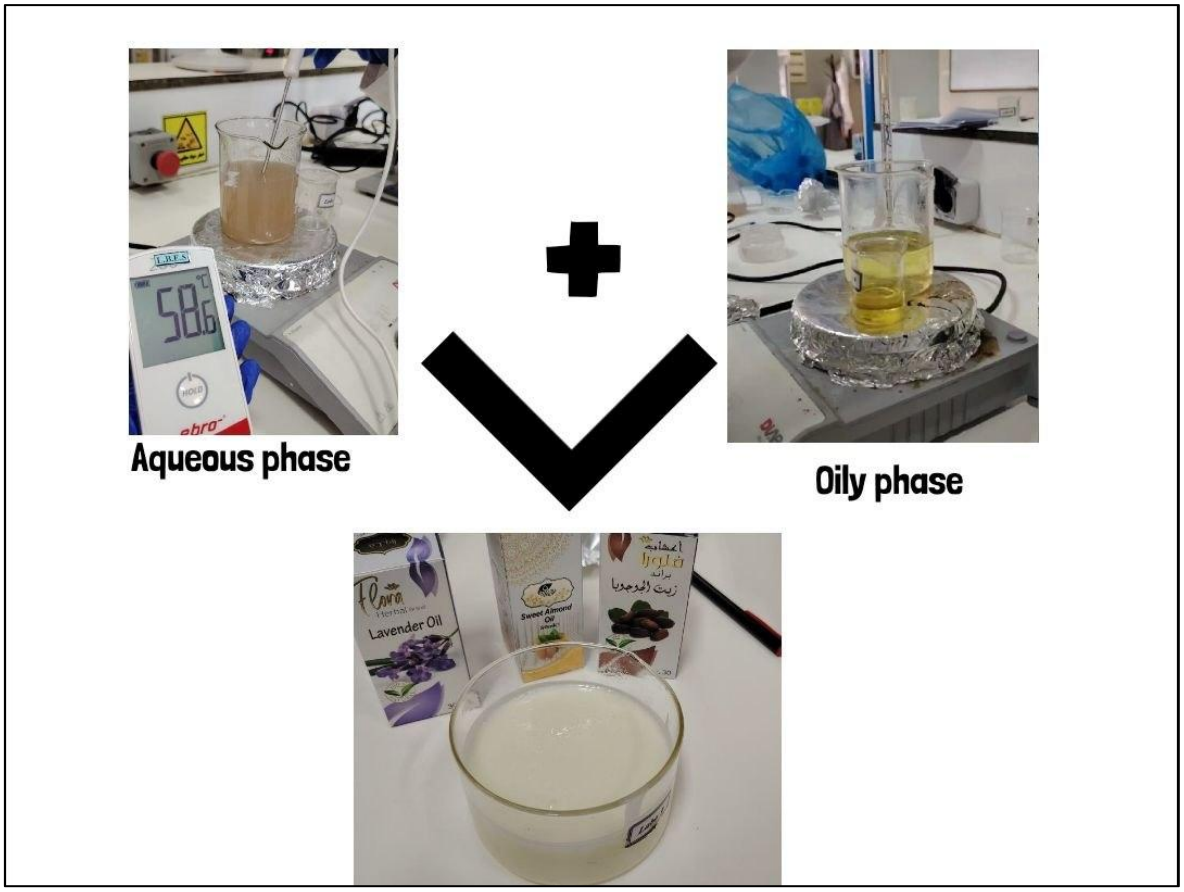


Figure 20. Mixing of the two emulsion phases

IV.4 Organoleptic characteristics

The cosmetic cream formulated with white polysaccharides extract had a smooth consistency, stable pH, uniform appearance, and a light beige to white color. It showed no signs of phase separation or film formation. These physical characteristics closely resemble those reported by **Shirajuddin et al. (2015)** in their cosmetic emulsions stabilized with natural polysaccharides, which also exhibited homogeneity and a favorable texture

Moreover, the absence of phase separation in our cream highlights the effectiveness of the formulation. It functions similarly to the plant-based polymers used in the study by **Shirajuddin et al. (2015),** helping to maintain the integrity between the oil and water phases.

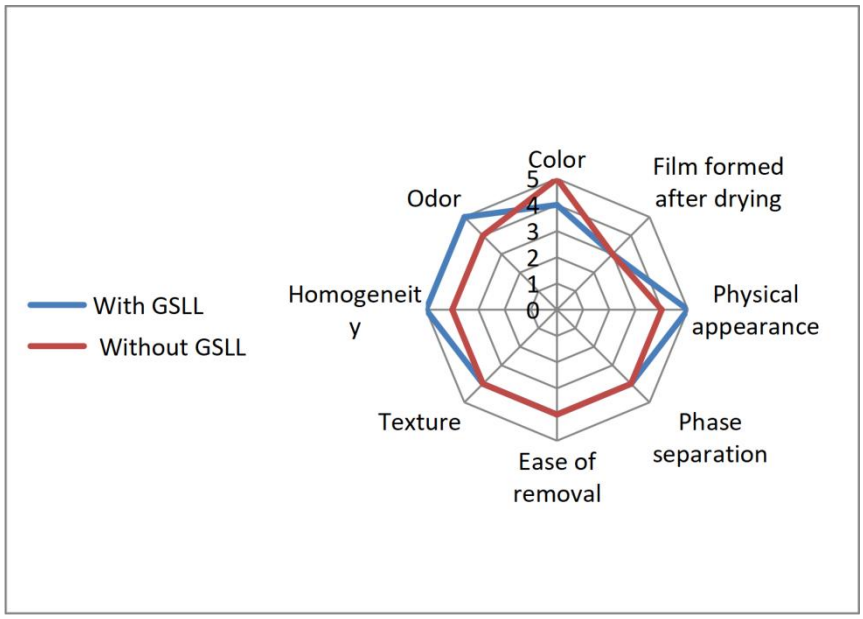
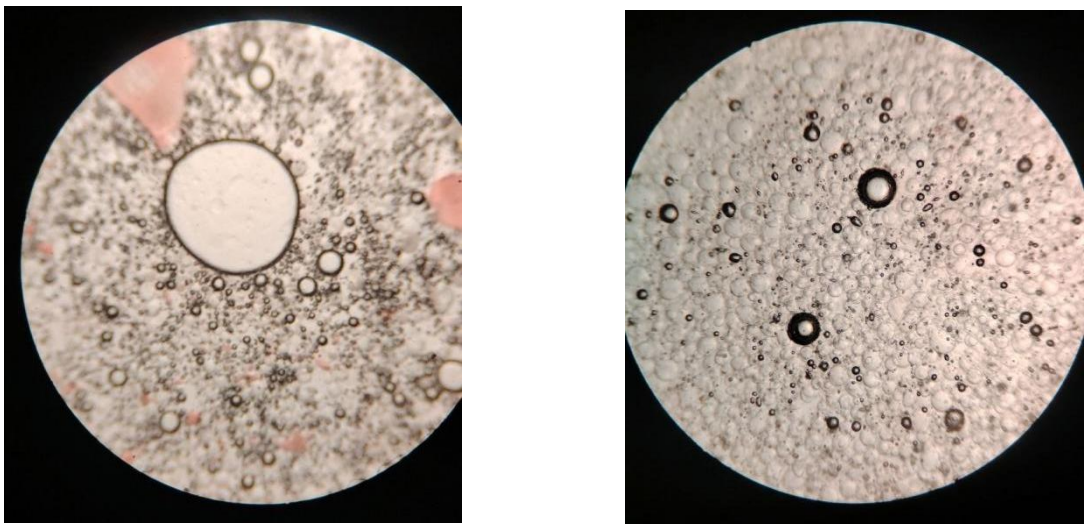


Figure 21. Radars representing the evaluation of the organoleptic properties of creams

IV.5 Physico-chemical characteristics

IV.5.1 Emulsion type

Figure 23 shows the microscopic images of the developed oil-in-water (O/W) creams, both with and without GSLL, after incorporating a hydrophilic red dye. In the formulation containing GSLL, the microscopic analysis revealed distinct red, gel-like aggregates formed by the interaction between the aqueous dye and the polymer matrix of the gum [GSLL]. This indicates that the gum [GSLL] successfully created a hydrogel network within the cream's continuous phase, enhancing viscosity and providing structural stability. On the other hand, the sample without gum GSLL displayed densely packed droplets without an organized network, lacking the stabilizing effect seen in the GSLL-stabilized version. This structural deficiency accounts for its lower viscosity and greater tendency to flow.



With GSLL **without GSLL**

Figure 22 The microscopic morphology of the formulated creams was observed under an optical microscope at a total magnification of 40× (using a 4× objective lens and a 10× ocular lens).

The presence of an oil-in-water (O/W) emulsion can facilitate wash-off with water, leading to improved customer compliance **(Barel et al., 2014).** This interpretation aligns with our findings and explains the ease of wash-off observed for all formulations in our study.

IV.5.2 Spreadability

The spreadability test (Figure 23) indicates how easily the cream can be applied to the skin.Our pharmaceutical and cosmetic formulation achieved a diffusion rate of 35.65 $g/cm^2\ s^{-1}$, notably higher than the typical baseline range of 22.03 to 26.79 g.cm/s commonly observed in standard herbal and topical formulations **(Senthil Raja, 2025).** This indicates superior diffusion properties, which contribute to smoother application, more uniform coverage across the skin, and broader surface distribution. As highlighted by **Senthil Raja (2025),** these advantages are critical to enhance user satisfaction while improving the overall efficacy and delivery of topical treatments."

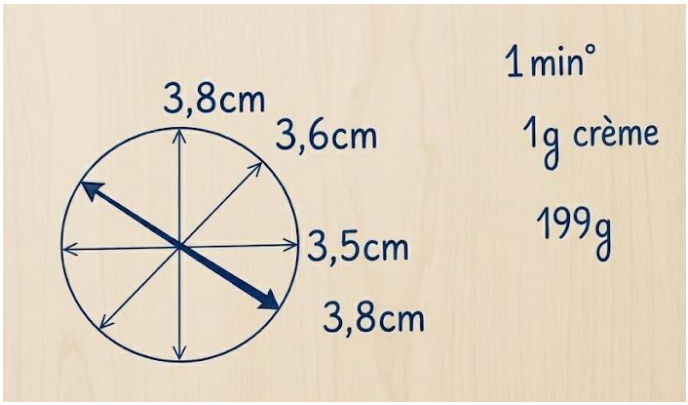


Figure 23 . Spreading capacity test

Spreadability plays a significant role in consumer acceptance and ensures uniform application of the cream over a larger area of skin. Spreadability also plays an important role in the delivery of a standard dose of a drug formulation to the skin and, consequently, in the efficacy of topical treatment **(Bhosale et al., 2024)**

IV.5.3 pH Values

The pH of the formulated cream, measured with a calibrated pH meter using a method adapted from **(Akhtar et al., 2010)**, was found to be 7.16. This value suggests that the emulsion has a near-neutral to mildly alkaline character. While normal, healthy skin typically maintains a slightly acidic pH between 4.5 and 5.5, a product with a pH of 7.16 is still regarded as safe and suitable for topical use, posing minimal risk of immediate skin irritation or compromise to the skin barrier. The formulation's relative neutrality likely stems from the inherent characteristics of the selected excipients in distilled water, as well as the lack of early acidic breakdown. Additionally, this result is consistent with observations reported by **McClements (2015)** which emphasize that a stable, neutral pH environment helps balance interfacial charges, reducing the likelihood of droplet coalescence and supporting the long-term physical stability of semi-solid emulsions.

IV.5.4 The Sun Protection Factor (SPF)

Using Mansur's formula on our experimental results gave an SPF value of:

SPF = 10 × 0,95296 ≈ 9,53

Table 16 Correlation between the erythemogenic effect (EE) and the intensity of radiation at each wavelength

λ(nm)	EE (λ) x I(λ)
290	1.004
295	0.993
300	0.959
305	0.950
310	0.943
315	0.921
320	0. 927

Spectrophotometric measurements showed the moisturizer provided an SPF of about 9.5. While not specifically designed as a sunscreen, this result highlights a notable inherent ability to absorb UVB radiation, supported by consistently high absorbance levels—nearly 1.00—across the full tested wavelength range.

Such a moderate level of sun protection is particularly beneficial in a daily-use moisturizer. This effect likely stems from the components in the emulsion's oil phase. Research indicates that vegetable oils and lipid-based excipients commonly used in cosmetic bases naturally filter ultraviolet light, contributing to the product's intrinsic SPF **(Korać & Khambholja, 2011).**

IV.5.5 Stability tests

Table 17 Result of previous stability tests

		Cream	
		Before	After
Centrifugation	Organoleptic characteristics	Ivory Smooth soft Refreshing scent	Heterogeneous Gelatinous Milky Liquid
	Organoleptic	Ivory	Heterogeneous

Thermal stress	characteristics	Smooth soft Refreshing scent		Yellowish Liquid	
	pH	6,22		6,90	
Freezing /Thawing	Organoleptic characteristics	Ivory Smooth soft Refreshing scent		Stable	
	pH	With GSLL	Without GSLL	With GSLL	Without GSLL
		5,98	6,22	6,11	6,32

❖ **Centrifugation**

The centrifugation test indicated significant physical instability in our moisturizing cream, evident through noticeable phase separation **(Figure24).**

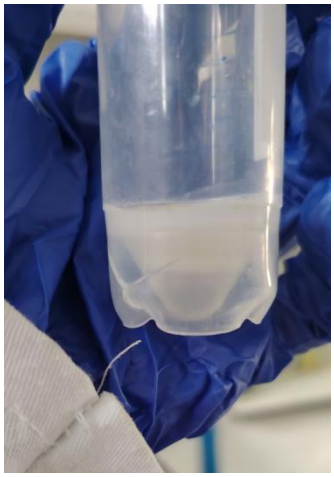


Figure 24 Visual aspect of the moisturizing cream after the centrifugation test,

Despite using GSLL—a highly effective emulsifier for oil-in-water emulsions—the applied centrifugal force was sufficient to disrupt the system's stability. This breakdown can be attributed to two primary mechanisms:

-Depletion-induced flocculation: The presence of mucilage, a polysaccharide, creates steric hindrance that pushes oil droplets into clusters, promoting coalescence and separation **(McClements, 2015).**

-Hydration competition: Mucilage absorbs free water within the formulation, reducing the water available to maintain the GSLL-stabilized gel network. This compromises the interfacial film, increasing vulnerability to mechanical stress **(Baeza et al., 2009)**

In contrast to **(Akhtar et al., 2010)** who observed stable, homogeneous creams, our product shows inadequate viscosity in the continuous phase, Addressing this issue would likely require incorporating a thickening agent to enhance structural resistance.**Table 17**

- **Thermal stress**

The heat stress test (**Figure 25**) showed that our moisturizing cream underwent full physical breakdown, evident from visible phase separation. In addition, the treatment led to a rise in pH, shifting from **6.21** prior to heating to **6.90** afterward (**Table 17**).

Figure 25 Phase separation appearance of moisturizing cream after thermal stress testing

Despite the inclusion of GSLL—an emulsifier known for its stability—exposure to high temperature compromised the emulsion through two main processes:

First, the heat weakened the crystalline gel structure created by GSLL, causing it to lose water and allowing oil droplets to merge **(McClements, 2015).**

Second, the pH increase indicates a chemical change in the mucilage component. As noted by **(Sciarini et al., 2009),** shifts in pH can alter the molecular configuration of polysaccharides, reducing their ability to maintain viscosity and stabilize the formulation.

Unlike **(Akhtar et al., 2010),** who observed greater heat resistance in their formulations, our continuous phase showed a reduction in viscosity, which, consistent with Stokes' law **(McClements, 2015).** led to faster sedimentation.

❖ **Freezing/Thawing**

The freeze/thaw test was conducted to evaluate the physical stability of our moisturizing cream under repeated and extreme temperature fluctuations. Unlike previous stress tests, the formulation containing both GSLL and mucilage demonstrated complete stability, with no evidence of phase separation observed **(Table 17).**

Moreover, both formulations maintained notably stable pH levels across all cycles. The control cream, which contained no mucilage, experienced only a small change, rising from an initial pH of 5.98 to 6.11 by the end of testing. Likewise, the cream formulated with mucilage demonstrated strong chemical stability, with pH increasing slightly from 6.22 to 6.32. Such minimal fluctuations indicate that both systems possess good buffering ability and retain their chemical integrity even when exposed to thermal stress.

The lasting uniformity is due to the ingredients' ability to provide both protection and structural support.

- Mucilage's cryoprotective effect: The polysaccharides present in mucilage have a strong ability to hold water, which helps limit the formation of large ice crystals when frozen. This, in turn, reduces mechanical stress on the interfacial layer surrounding droplets **(Gollavilli et al., 2020).**
- The emulsifying network formed by GSLL maintained its structural flexibility and stability around oil droplets, effectively inhibiting coalescence during thawing **(McClements, 2015).**

Our results are consistent with those of **(Akhtar et al., 2010)** ,who found that combining hydrophilic natural polymers with structured surfactants enhances the stability of oil-in-water emulsions during freeze-thaw cycles. This study further confirms that observation, demonstrating that the cream retains good physical and chemical stability over time, including under significant variations in climate conditions **(McClements, 2015).**

IV.5.6.Viscosity

Comparative analysis of the two curves shows that the moisturizing cream formulated with GSLL exhibits significantly higher viscosity values across the entire shear range studied than the reference cream without GSLL. **Figure 26**

-These observations are consistent with the studies by **(Sciarini et al., 2009),** and **(Akhtar et al., 2010),** which highlight the essential role of Galactomannan emulsifiers in improving the viscosity and texture of hydrophilic creams enriched with natural polysaccharides. The structure stabilized by GSLL also explains the excellent resistance to freeze-thaw cycles.

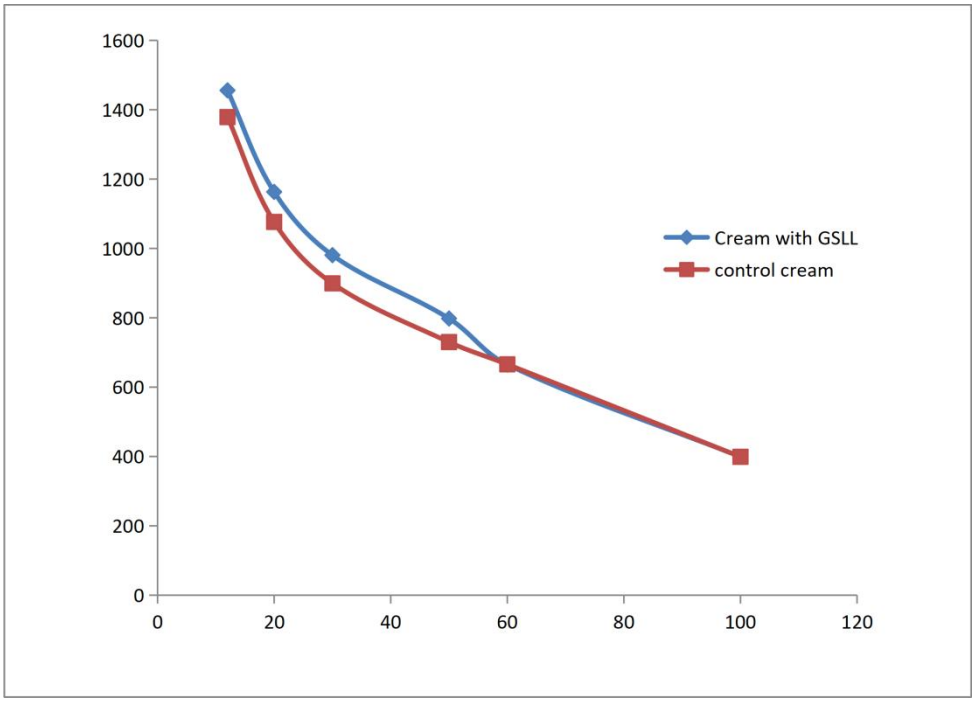


Figure 26 Viscosity profiles as a function of shear rate for the moisturizing cream containing GSLL compared to the control cream (without GSLL).

IV.6. Microbiological Quality Control of the cream

Microbiological quality control analyses were conducted on the cream sample to evaluate its hygienic quality and microbiological safety through the detection and enumeration of bacterial and fungal contaminants. The obtained results are presented in the following tables.

IV.6.1. Total bacterial count

- **Total bacterial count 24 hours after cream preparation**

According to the results presented in **Table 18**, the microbiological assessment conducted 24 hours after cream preparation demonstrated compliance with stringent cosmetic safety criteria, with no bacterial growth observed in the majority of dilutions tested (original, 10^{-1}, and 10^{-2}). A single instance of microbial growth ,49 colonies in one plate (P2) at the 10^{-3} dilution , was

considered an isolated case of *in vitro* contamination rather than a sign of inherent product compromise. Since all countable plates contained **fewerthan30 colonies**, the obtained counts were below the recommended range for reliable quantitative enumeration. Consequently, **CFU/g values were not determined**, and the results were interpreted qualitatively based on the observed microbial growth.

Table 18. Monitoring of bacterial contamination in cream samples after 24 hours

Dilution	Plate Nº	Colony shape	Colony color	Number of colony types	Bacterial count	Observation
Original	P1	-	0	0	**0**	No growth
	P 2	-	0	0	**0**	No growth
	P 3	-	0	0	**0**	No growth
10^{-1}	P1	-	0	0	**0**	No growth
	P 2	-	0	0	**0**	No growth
	P3	-	0	0	**0**	No growth
10^{-2}	P1	-	0	0	**0**	No growth
	P2	-	0	0	**0**	No growth
	P 3	-	0	0	**0**	No growth
10^{-3}	P1	-	0	0	**0**	No growth
	P 2	Circular , smooth	white	1	49	Moderate growth (49 colonies)
	P 3	-	-	0	**0**	No growth

P:Plate **Incubation temperature: 37°C**

❖ **Total bacterial count 7days after cream preparation**

After one week of storage, the total aerobic mesophilic bacterial count reached **6.9×10^3** CFU/g, exceeding the maximum acceptable limit (M = 10^3 CFU/g) established by the

Official Journal of the People's Democratic Republic of Algeria N°. 16 (2020). Therefore, the microbiological quality of the formulated cream was considered unsatisfactory (table 19).

Table 19 . Monitoring of bacterial contamination in cream samples after 7 days

Dillution	Plate No	Colony shape	Colony color	Number of colony types	Bacterial count	Observation
Original	**P1**	circular and irregular, smooth /mucoid	white	1	65	Moderate growth
	P2	circular and irregular, smooth /mucoid	white	1	59	Moderate growth
	P3	circular and irregular, smooth /mucoid	white	1	84	High growth
10^{-1}	**P1**	Circular,smooth	white	1	**4**	Low growth
	P2	Circular,smooth	white	1	**10**	Low growth
	P3	Circular,smooth	white	1	**6**	Low growth
10^{-2}	**P1**	Circular,smooth	white	1	**1**	Very low growth
	P2	Circular with transparent halo	white	2	**14**	Mixed colonies observed
	P3	Circular,smooth	white	1	**6**	Low growth
10^{-3}	**P1**	Circular,smooth	white	1	**6**	Low growth
	P2	Circular,smooth	white	1	**5**	Low growth
	P3	Circular,smooth	white	1	**3**	Low growth

P:Plate

- **Total bacterial count 16 days after cream preparation**

As shown in **Table 20**, bacterial growth was observed in several plates across the dilution series. The colonies exhibited different morphologies, including circular, irregular, convex, and confluent forms, and were predominantly white to creamy white in color. Several plates showed heavy bacterial growth recorded as too numerous to count (TNTC), while others presented low to moderate counts.

The results showed substantial variability among replicate plates, and the expected reduction in colony counts across successive dilutions was not consistently observed. As a result, accurate quantitative enumeration was not achievable, and **CFU/g** values were therefore **not calculated.**

Table 20. Monitoring of bacterial contamination in cream samples after 16 days.

Dilution	Plate N°	Colony shape	Colony color	Number of colony types	Bacterial count	Observation
Original	**P1**	Small circular colonies and convex creamy colony	white	2	15	Moderate bacterial growth observed
	P2	**-**	**-**	0	0	No growth
	P3	-	-	0	0	No growth
10^{-1}	**P1**	Circular,smooth, slightly convex	White to cream	1	TNTC*	Heavy bacterial growth
	P2	Circular, smooth colonies creamy colonytranslucent colony peripheral irregular growth	White,creamy White, pale yellow and translucent	4	10	Mixed bacterial growth
	P3	Irregular confluent colonies	Creamy white	1	TNTC*	Heavy bacterial growth
10^{-2}	**P1**	Confluent Irregular growth	Creamy white (beige)	1	TNTC*	Heavy growth covering most of the plate
	P2	Confluent Irregular growth	Creamy white (beige)	1	TNTC*	Heavy growth covering most of the plate
	P3	Small circular colonies and one medium irregular creamy colony	White and creamy white	2	43	Mixed bacterial growth

10^{-3}	P1	Confluent Irregular growth	White and creamy white	2	TNTC	Confluent growth observed
	P3	Confluent Irregular growth	White and creamy white	2	TNTC	Heavy growth covering most of the plate
	P3	Confluent Irregular growth	White and creamy white	2	TNTC	confluent growth

P:Plate, **TNTC** = Too Numerous To Count

❖ **Total bacterial count 21 days after cream preparation**

As shown in **Table 21**, bacterial growth was generally low and irregularly distributed across the dilution series. The observed colonies were predominantly small, circular, and white to creamy-white in color, although some irregular, spreading, and punctiform colonies were also detected. Several plates showed no growth, whereas others exhibited low colony numbers or growth recorded as too numerous to count (TNTC).

Considerable variability was observed among replicate plates, and colony counts did not consistently decrease with increasing dilution as expected. Consequently, reliable quantitative enumeration was not possible, and CFU/g values **were not determined**. Overall, the low colony numbers observed on most countable plates suggest a relatively **lowbacterial load** in the analyzed cream sample, although the irregular distribution of colonies limited accurate quantitative assessment.

Table 21. Monitoring of bacterial contamination in cream samples after 21 days

Dillution	Plate No	Colony shape	Colony color	Number of colony types	Bacterial count (CFU/ml)	Observation
Original	P1	Small circles , overlapping colony	White	2	18	Low growth observed
	P2	-	-	0	-	No growth
	P3	-	-	0	-	No growth
10^{-1}	P1	Small circles confluent growth	White and beige	1	TNTC*	Low growth bacterial
	P2	-	-	0	0	No growth
	P3	Two irregular spreading	White creamy and beige	2	3	Three countable colonies

		colonies and one small circular colony				observed
10^{-2}	P1	Two small circles	Transparent , white	2	2	Growth consists of small
	P2	Small and irregular colonies	white	1	11	Small, scattered colonies with low growth.
	P3	Small circular colonies with confluent growth	white	1	TNTC*	colony count not
10^{-3}	P1	Small circles and confluent growth	White Creamy	1	45	Very low bacterial growth with sparse colonies.
	P2	Could-shaped colony morphology	beige	1	1	Low bacterial growth
	P3	translucent punctiform colonies, and separate creamy	Beige and white	2	2	Mixed growth pattern with a large area of small

P:Plate, TNTC = Too Numerous To Count

- **Discussion of the Total Aerobic Mesophilic Bacterial Count**

The changes in the total aerobic mesophilic bacterial count over the 21-day storage period indicate shifts in bacterial survivaland growth within the cream. The minimal bacterial presence right after preparation points to good hygiene during manufacturing, consistent with **Kallings *et al.* (1969),** who stressed the importance of high initial microbiological standards in cosmetic products. However, by day 7, bacterial levels had risen beyond the threshold set by Algeria's Official Journal N°. 16 (2020), suggesting that residual microorganisms began to multiply during storage. This quick escalation in microbial load, particularly in unpreserved or naturally derived formulations following an initial stable phase, mirrors findings reported by **Cobzaru *et al.* (2025)** in their study of preservative-free creams.

Bacterial growth was still observed at 16 and 21 days, but the inconsistency between replicate plates made accurate quantification unfeasible. This ongoing but variable microbial

presence aligns with findings from regional market studies, including **Techaoei (2017)** in Thailand and **Norouz-zadeh *et al.* (2014)** in Iran, which reported widespread bacterial and fungal contamination in commercial foundation and skincare creams, largely attributed to insufficient microbial control after formulation.

These results underscore the need for robust preservation systems and adherence to good manufacturing practices (GMP) to ensure microbiological quality during storage. The findings align closely with **Jairoun *et al.* (2020),** who emphasized the significant disparity between adequate chemical preservation and the risk of microbial contamination in cosmeceuticals. They also reinforce the conclusions of **Althulthi *et al.* (2023),** who showed that long-term microbiological failure in cosmetic products is largely due to declining preservative effectiveness over time.

IV.6.2. Total fungal count

❖ **Total fungal count 24 h after cream preparation**

The results presented in **Table 22** showed a very low fungal contamination level in the formulated cream. Yeast growth was observed only in a few plates, with colony counts ranging from 0 to 3 CFU. The highest count was recorded in one plate of the second dilution, whereas several plates showed no growth. Mold growth was also limited, with only isolated colonies detected in some plates and complete absence of growth in most others.

Overall, the low numbers of yeast and mold colonies indicate **a weak fungalload** in the analyzed samples. Since all countable plates contained **fewer than 30** colonies, the counts were considered **statisticallyinsufficient** for accurate quantitative enumeration. Therefore, the calculation of CFU/g was not considered reliable for quantitative interpretation, and the results were evaluated qualitatively based on the observed fungal growth.

Table 22. Monitoring of fungal contamination in cream samples after 24 hours

Dilution	Plate Nº	Colony shape	Colony color	Number of colony types	Total count	Observation
Original	P1	M:Cottony	white	M:1 Y:0	M:1 Y:0	Low growth
	P 2	M:Cottony Y:Smooth	M:white Y:Creamy	M:1 Y:1	M:1 Y:1	Very dense molds growth covering most of the plate, non-countable (contaminated)
	P 3	M:Cottony	Y:Creamy	M:1	M:1	Moderate growth with

		Y:Smooth	white M: red	Y:4	Y:4	isolated smooth colonies
10^{-1}	P1	Y:Smooth	Y: White	Y:1	Y:TNTC	Moderate to dense growth with colonies of different sizes
	P 2	M: Cottony Colonies Y:Smooth	Y:Creamy white , M:light orange	M:1 Y:1	M:1 Y:4	Moderate molds growth, countable colonies
	P3	Y: Smooth	beige	M:0 Y:1	M:0 Y:1	Dense growth forming a single large colony
10^{-2}	P1	Y: Small circle	white	M:0 Y:1	M:0 Y:3	Very low growth
	P2	Y: Small circle	white	M:0 Y:1	M:0 Y:1	Low growth, single Isolated colony
	P 3	-	-	M:0 Y:0	M:0 Y:0	No growth
10^{-3}	P1	Y: irregular yeast powdery-like	Creamy white	M:0 Y:1	M:0 Y:TNTC	Dense powdery-like growthcoveringalmost the entire plate
	P 2	-	-	M:0 Y:0	M:0 Y:0	No growth observed
	P 3	Y:Irregular	White	M:0 Y:1	M:0 Y:TNTC	Very low Growth (contaminated)

P:Plate, **M:**Molds, **Y:**Yeast, **TNTC** = Too Numerous To Count

❖ Total fungal count 16 days after cream preparation

As shown in **Table 23**, fungal growth was dominated by yeasts, which were detected in most of the inoculated plates. Several plates exhibited yeast growth recorded as too numerous to count (TNTC), while the countable plates showed between 1 and 6 colonies. The yeast colonies displayed various morphologies and colors, ranging from white and creamy white to beige and dark brown. Some plates were classified as TNTC because the colonies were not sufficiently isolated for reliable enumeration, likely due to colony spreading and overlapping growth.

In contrast, mold growth was limited, with only a few isolated colonies observed and no growth detected on most plates. The mold colonies were mainly white and exhibited a cottony appearance. Overall, yeasts were the predominant fungal contaminants, whereas molds occurred only sporadically.

Since the countable plates contained **fewer than 30colonies**, accurate quantitative enumeration was not possible; therefore, CFU/g values were not calculated.

Table 23. Monitoring of fungal contamination in cream samples after 16 days

Dilution	Plate Nº	Colony shape	Colony color	Number of colony types	Total count	Observation
Original	P1	Y: irregular colony unncountable	white	M:0 Y:1	M:0 Y:TNTC	Low growth
	P 2	Y:Large irregular colony	Y:Dark brown to black	M:0 Y:1	M:0 Y:TNTC	Heavy growth with extensive surface coverage (contaminated)
	P 3	Y:irregular colony	white	M:0 Y:1	M:0 Y:4	Low growth
10^{-1}	P1	Y: irregular colony	White	M:0 Y:1	M:0 Y:5	Very low growth
	P 2	Y: Small Point colonies	White	M:0 Y:1	M:0 Y:6	Very low growth
	P3	M:cottony Y: irregular colonies unncountable	Y:White , M: beige	M:1 Y:1	M:1 Y:TNTC	Moderate growth
10^{-2}	P1	Y:Small irregular colony	beige	M:0 Y:1	M:0 Y:1	Low growth
	P2	Y: irregular colonies unncountable M:cottony White	Y:Creamy White M:white	M:1 Y:1	M:1 Y:TNTC	Dense growth with colonies that are difficult to distinguish
	P 3	Y:Scattered Small colonies unncountable	white	M:0 Y:1	M:0 Y:TNTC	Moderate growth with scattered colonies
10^{-3}	P1	Y:small circular colonies irregular colony uncountable	Creamy White	M:0 Y:2	M:0 Y:TNTC	Moderate growth with clearly isolated colonies
	P 2	Y:Scattered small colonies irregular	Creamy White	M:0 Y:1	M:0 Y:2	Weak growth with a low number of colonies
	P 3	Y: irregular colonies unncountable	Creamy white , light brown	M:0 Y:2	M:0 Y:TNTC	Moderate growth

P:Plate, **M:**Molds, **Y:**Yeast, **TNTC** = Too Numerous To Count

❖ **Total fungal count 21 days after cream preparation**

As shown in **Table 24,** fungal growth remained generally low throughout the dilution series. Yeast colonies were detected more frequently than molds, with counts ranging from 1 to 8 colonies on the countable plates. The yeast colonies exhibited different morphologies, including small circular, irregular, and scattered forms, and varied in color from white and creamy white to beige. One plate showed growth recorded as TNTC due to extensive colony coverage, which may have resulted from colony spreading and overlap rather than exclusively from a high microbial concentration.

Mold growth was limited, with only a few isolated colonies observed on some plates and complete absence of growth on others. The mold colonies were mainly cottony in appearance and displayed white, beige, or light orange pigmentation.

Since all countable plates contained **fewer than 30 colonies**, accurate enumeration was not possible. Nevertheless, the low colony numbers and the predominance of isolated colonies suggest a low fungal load in the analyzed cream sample after 21 days of storage.

Table 24. Monitoring of fungal contamination in cream samples after 21 days

Dilution	Plate Nº	Colony shape	Colony color	Number of colony types	Total count	Observation
Original	P1	M:cottony	M:beige	Y :0 M:1	M:1 Y:0	Very weak peripheral growth
	P 2	Y: irregular colony and 1small round	Y:beige	Y:1 M:0	Y:2 M:0	Moderate peripheral growth
	P 3	M:Cottony colony Y: small circle	Y:Cremy M: light orange	Y:1 M:1	Y:3 M:2	Low growth with a pigmented mold colonypigmentation
10^{-1}	P1	M: Cottony dark brown circular Y:small circle ,transparent	Y:Creamy white M:dark brown	Y:2 M:1	Y:4 M:1	Low growth Few isolated colonies

	P 2	Y:irregular colony	white	Y:1 M:0	Y:tntc M:0	Heavy growth covering most of the plate
	P3	M:Cottony circular Y:small round	Y: white M:Beige with light brown center	M:1 Y:1	M:1 Y:1	very low growth colonies
10^{-2}	P1	M:Cottony, half circle	M:Beige	Y:0 M:1	Y:1 M:0	Low growth of a visible colony at the periphery of the plate
	P2	Y:small circle colonies	Y:white	Y:1 M:0	Y:6 M:0	Very low growth at surface to plate.
	P 3	,half circle And red round Y: circlular	M:Beige, red Y: white	Y :1 M :2	Y:1 M:2	Low growth and widely spaced, disparate colonies
10^{-3}	P1	Y: small scattered circles	Y:beige	Y:1 M:0	Y:8 M:0	Low growth , small scattered circles
	P 2	Y:transparn t round With 3 small circles	Y: round transparent with white center	Y:1 M:0	Y:tntc M:0	Moderate growth of overlapping colonies with small circular colonies.
	P 3	M:cottony colonies Y:irregular colony	M:dark ,circular Y:white irregular + transparent with green center	Y:2 M:2	Y:2 M:2	Moderate growth on surface (contaminated)

- **Discussion of the totalfungal count**

Fungal analysis showed consistently low levels of contamination throughout the storage period, with yeasts being more prevalent than molds. This trend is likely due to yeasts' higher tolerance for the physicochemical environment found in cosmetic emulsions, while molds typically need more conducive conditions to grow.

Such differences in fungal survival and adaptability in personal care products are consistent with observations reported by **Techaoei (2017).** Although some yeast samples reached counts too numerous to count (TNTC), the majority of readable plates had fewer than 30 colonies, indicating that overall fungal growth remained minimal during storage.

The limited mold growth noted could be attributed to the inhibitory properties of the formulation's components and the influence of storage conditions. It is well established that formulation design and preservative effectiveness play key roles in controlling baseline microbial increases. For example, **Althulthi *et al.* (2023)** pointed out how preservative efficiency is vital for preserving cosmetic product quality, whereas **Jairoun *et al.* (2020)** stressed the need for a careful balance between adequate preservation and minimizing microbial contamination. Additionally, evidence that certain natural ingredients in formulations can aid microbial control, even under unfavorable conditions, aligns with recent findings by **Cobzaru *et al.* (2025),** who explored preservative-free cosmetic creams using plant extracts to achieve microbiological stability.

The low levels of fungal contamination observed point to acceptable fungal quality and indicate adherence to the microbiological standards set out in the Official Journal of the People's Democratic Republic of Algeria N°. 16 (2020). Maintaining compliance with these stringent limits for commercial cosmetics has long been a public health priority, a concern first highlighted by **Kallings *et al.* (1969)** in their study on the contamination of medical and personal care products. This also underscores the continued importance of monitoring the market, as shown by **Norouz-zadeh *et al.* (2014),** who emphasized microbial testing of foundation creams to ensure consumer safety.

IV.6.3. Detection of the pathogen bacteria

The initial test took place after **six days**, on **19 April 2026**, followed by the second test thirteen days later, on **26 April 2026**. According to the official laboratory findings recorded in the analysis report, no trace of the specified standard pathogenic bacteria was detected during either testing phase **(Table 25 and Table 26).**

The critical strains involved are *Escherichia coli* (in accordance with standard NA ISO 21150), *Pseudomonas aeruginosa* (per NA ISO 22717), and *coagulase-positiveStaphylococci* (as specified in NA ISO 22718).

The cream's capacity to withstand microbial growth and comply with national standard criteria (m/M) for nearly two weeks after formulation highlights **the success of its biological design.** The absence of pathogenic bacteria in samples collected at both 6 and 13 days can be

attributed to the strict hygiene and sterilization protocols maintained in the lab during preparation. This suggests the manufacturing process began in a microbiologically uncontaminated state.

The complete lack of initial contamination supports the core claims made by **Kallings *et al.* (1969),** who stressed that following strict sanitary procedures at the beginning of production is crucial for preventing hardy pathogens from entering medical and cosmetic products. When manufacturing starts under entirely clean conditions, the likelihood of microbial growth later on decreases dramatically, a finding reinforced by recent evaluations of quality control in cosmetics **(Althulthi *et al.,* 2023).**

Moreover, the formulation's ability to remain free of common pathogenic microorganisms for almost two weeks underscores its strong structural integrity. This is especially important because commercially available cosmetic products often face regulatory issues due to inadequate preservation or initial contamination. For example, research by **Jairoun *et al.* (2020)** revealed a recurring issue in commercial cosmeceuticals, insufficient preservation leading to microbial contamination, including harmful bacteria such as *Pseudomonas aeruginosa* and *Staphylococci.* Comparable findings emerged from regional studies, including **Techaoei's (2017)** survey in Northern Thailand and **Norouz-zadeh *et al.***'s **(2014)** work in Iran, both of which reported widespread bacterial and fungal contamination in skincare and foundation products, typically linked to poor manufacturing conditions or ineffective preservative systems.

Unlike the evident vulnerabilities observed in market products, the developed cream effectively prevented colonization by pathogenic organisms. This strong protective capacity aligns with the results reported by **Cobzaru *et al.* (2025),** who found that natural bioactive formulations , particularly those enriched with powerful plant-derived extracts, can possess inherent antimicrobial qualities. These properties enable them to meet microbiological standards without relying on conventional synthetic preservatives. As a result, the combination of rigorous aseptic preparation methods and the cream's biologically informed formulation creates a reliable defense against microbial contamination, supporting both its safety profile and compliance with shelf-life requirements.

Table 25. Temporal microbiological evaluation of the moisturizing cream samples after 6 days of formulation.

Parameter	Sample					Standard		Method
	1	2	3	4	5	m	M	
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 21150(NA 14808)
Pseudomonas aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22717
Coagulase $^{+}$ Staphylococci	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22718(NA 14809)

Table 26. Temporal microbiological evaluation of the moisturizing cream samples after 13 days of formulation.

Parameter	Sample					Standard		Method
	1	2	3	4	5	m	M	
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 21150(NA 14808)
Pseudomonas aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22717
Coagulase $^{+}$ Staphylococci	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22718(NA 14809)

IV.7 Biological Activities of The moisturizer cream

IV.7.1 Antioxydant activities

The antioxidant activity of the developed moisturizing cream was assessed using the DPPH radical scavenging method. The results showed an IC_{50} value of 1.76 ± 0 mg/mL for the cream,

whereas ascorbic acid, serving as the positive control, exhibited a much lower IC_{50} value of 0.095 ± 0.007 mg/mL, indicating stronger antioxidant activity**.**

The lower IC_{50} value of ascorbic acid highlights its role as a strong, pure antioxidant standard. In comparison, the higher IC_{50} value observed for the cream is typical for topical formulations. This difference arises because the active plant components—specifically mucilage—are dispersed and diluted within a complex mixture of water, lipids, and the GSLL emulsifier, none of which have radical-scavenging properties **(McClements, 2015).**

Nevertheless, an IC_{50} value of 1.76 mg/mL shows that the cream maintains significant and functional antioxidant activity, suggesting that neither the formulation procedure nor the organized GSLL structure interfered with or concealed the mucilage's bioactive functional groups **(Ji et al. (2024)).**

These results indicate that adding natural polysaccharide extracts to stable oil-in-water emulsions can yield topical formulations with significant potential to protect the skin locally from oxidative stress **(Di Mambro & Fonseca, 2005; Dini & Laneri, 2021).**

IV.7.2 Antibacterial Activity

The antibacterial activity of the The moisturized cream was evaluated against E.coli and Staphylococcus aureus using the disk diffusion method. As shown in **Table 27.**

Experimental results indicate that the cream developed exhibits relative inhibitory activity, the intensity of which varies depending on the concentration of the incorporated active ingredients.

Table 27. Antibacterial Activity

Strains	Concentration (mg/ml)	Extract(cm)	Gentamycin (cm)
E. coli	1	1.86 ±0.41	2.66 ±0.66.
	0.5	1.43 ±0.37	
	0.25	1.33 ±0.45	
Staphylococcus aureus	1	1.53 ±0.47	2.70 ±0.10
	0.5	1.43 ±0.49	
	0.25	1.36 ±0.25	

Although this antibacterial action it has a good action is less potent than that of the reference antibiotic **(Pandey et al., 2014),** it demonstrates that the emulsion structure, particularly the moisturizer cream, does not impede the diffusion of active molecules from the mucilage into the agar medium. According to **Ribeiro et al. (2015)** the successful integration of natural polysaccharides into moisturizing cream formulations allows for the preservation of both a protective barrier effect and local antimicrobial activity, making it advantageous for topical use.

Conclusion

Conclusion

This study primarily investigated the functional and dermocosmetic potential of water-soluble galactomannan extracted from the seeds of *Leucaena leucocephala* collected in El Oued province. Experimental results demonstrated that hot water maceration is an effective and environmentally friendly extraction method, yielding a substantial 11.66% output. Chemical analysis revealed a high carbohydrate content, with total sugars accounting for 97.7%, along with the presence of co-extracted proteins and polyphenols. These components worked together synergistically, endowing the crude extract with strong thickening and stabilizing capabilities properties particularly valuable in topical product formulations.

An oil-in-water (O/W) emulsion containing 5% of this plant-derived mucilage produced a high-quality cosmetic cream with excellent sensory properties. The biopolymer effectively strengthened the hydrogel structure in the continuous phase, substantially boosting viscosity while maintaining good spreadability on the skin. Clinical testing showed a skin-friendly pH of 7.16, indicating strong compatibility, along with inherent sun protection offering an SPF of about 9.53. Although the formulation showed signs of phase separation when exposed to intense heat and centrifugation, it remained highly stable through repeated freezing and thawing cycles.

Microbiological evaluation showed low fungal contamination throughout storage, with yeasts predominating over molds. The total aerobic mesophilic bacterial population exhibited fluctuations during storage, including an increase after 7 days that exceeded the limit established by the Official Journal of the People's Democratic Republic of Algeria N°. 16 (2020). **No pathogenic microorganisms**, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *coagulase-positive Staphylococci*, were detected in any sample in agreement with the national standards. Overall, the formulated cream demonstrated **good resistance** to pathogenic microbial contamination and **generally satisfactory microbiological quality**, although improvements in preservation may be required to ensure full compliance during storage.

Going forward, several avenues are worth pursuing to build on these encouraging findings and support industrial adoption. Future efforts should aim to refine the formulation by combining galactomannan with other natural texturizing agents to enhance stability and prevent phase separation under intense physical conditions. Complementing this, *in vivo* clinical studies assessing reductions in transepidermal water loss, along with extended aging tests, will offer a clearer formulation of the product’s long-term hydrating efficacy. This work paves the way for new economic opportunities in utilizing locally adapted plant resources to develop a safe, sustainable bio-stabilizer for Algeria’s cosmetics sector.

References

References

1. Abdelkader, H. A., et al. (2023). Formulation and evaluation of galactomannan- based moisturizing creams for cosmetic applications. Journal of Cosmetic Dermatology, 22(5), 1120–1130.
2. Adeyemi, O. S., Fawole, O. A., & Adewusi, E. A. (2021). Plant-based polysaccharides as functional ingredients in cosmetic formulations: A review. International Journal of Biological Macromolecules, 183, 2195–2205.
3. Aernan, P.T., Odo, J.I., Aondofa, T.J., Anietie, D.J., Iqbal, M.N., 2023. Investigation of Bacteria and Fungi Associated with Onion (Allium cepa) Bulbs Rot Purchased from Markets in Makurdi, Central Nigeria. Int. J. Nanotechnol. Allied Sci., 7(2): 14–23.
4. Agi, V.N., Ollor, O.A., Azike, C.A., Onwuasoanya, C.V., 2023. Isolation and identification of microorganisms from makeup brushes in Rivers State University Nigeria and its environs. J. Adv. Microbiol., 23(3): 41-50.
5. Ai, W., Fang, Y., Xiang, S., Yao, X., Geng, F., & Bandara, N. (2015). Protein/polysaccharide electrostatic complexes and their applications in stabilizing oil-in-water emulsions. *Journal of Nutritional Science and Vitaminology*, *61*(Supplement), S168–S169.
6. Ai, W., Fang, Y., Xiang, S., Yao, X., Geng, F., & Bandara, N. (2015). Protein/polysaccharide electrostatic complexes and their applications in stabilizing oil-in-water emulsions. *Journal of Nutritional Science and Vitaminology*, *61*(Supplement), S168–S169.
7. Akhtar, N., Khan, H. M. S., Gulfishan, Rasool, F., Khan, S. M. S., & Mahmood, T. (2010). Formulation and *in vitro* evaluation of a cosmetic emulsion containing apple juice extract. *Asian Journal of Chemistry*, *22*(9), 7235–7242.
8. Al- Jawadi, Q. A., et al. (2021). Extract galactomannan from *Leucaena leucocephala* plant seeds and its characterization. Journal of Physics: Conference Series, 1879(2), 022071.
9. Al- Jawadi, Q. A., et al. (2024). Extraction and characterization of galactomannan from seeds of *Leucaena leucocephala*. Advanced Materials Research, 1134, 213–219
10. Al-Nabulsi, A., et al. (2023). Microbial safety and stability of cosmetic emulsions formulated with natural biopolymers. Journal of Cosmetic Science, 74(2), 145-158.
11. Althulthi, R. F., Moman, R. M., Ghalbun, A. A., & Alsagher, M. R. (2023). Evaluation of Bacteriological Quality and Preservatives Efficacy of Cosmetics. Scholars Academic Journal of Biosciences, 11(12), 442–451.
12. Article on Analysis of the physical and chemical characteristics of stored and fresh Leucaena leucocephala seeds (2026). Food Research International (ScienceDirect). Amanda Graziela

Gonçalves Mendes , Viviane da Silva Sousa Almeida , Daniele Gomes Cassias Rodrigues , Tonicley Alexandre da Silva

13. Azani, N., Babineau, M., Bailey, C. D., Banks, H. I., Barbosa, A. R., Pinto, R. B., Boatwright, J. S., Borges, L. M., Brown, G. K., Bruneau, A., Candido, E., Cardoso, D., Chung, K. F., Clark, S., Conceição, A. S., Crisp, M., Cubas, P., Delgado-Salinas, A., Devisetty, U. K., . . . LPWG. (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon*, *66*(1), 44–77..

14. Baeza, R., Carrera Sanchez, C., Pilosof, A. M. R., & Rodriguez Patino, J. M. (2009). Interfacial dynamic properties of whey protein concentrate/polysaccharide mixtures at neutral pH. *Food Hydrocolloids*, *23*(5), 1253–1262

15. Barel, A. O., Paye, M., & Maibach, H. I. (Eds.). (2014). Handbook of cosmetic science and technology (4th ed.). CRC Press.

16. Barel, A. O., Paye, M., & Maibach, H. I. (Eds.). (2014). Handbook of cosmetic science and technology (4th ed.). CRC Press

17. Belhadj, A., Allache, F. D., Boulghobra, N., & Mebrek, N. (2023). Botanical diversity of arid steppe in South East of Algeria (Biskra). Journal of Ecological Engineering, 24(1), 120–134

18. Benaoun, F., Delattre, C., Boual, Z., Ursu, A. V., Vial, C., & Gardarin, C. (2017). Structural characterization and rheological behavior of a heteroxylan extracted from Plantago notata Lagasca (Plantaginaceae) seeds. *Carbohydrate Polymers*, 175, 96-104.

19. Benbrahim, K., Eddoud, A., Benameur-Saggou, H., & Chennouf, R. (2021). Bio-ecological characterization of the flora of succession of the abandoned cereal areas in the region of Hassi Ben Abdallah-Ouargla (Sahara, Algeria). Algerian Journal of Arid Environment, 10(2), 91–102.

20. Bendjelil, A., et al. (2023). Agroforestry systems based on leguminous forage crops in arid and semi- arid regions of Algeria. Agricultural Systems, 204, 103642.

21. Bendjelil, A., et al. (2023). Floristic and ecological characterization of the arid Saharan and pre- Saharan vegetation in Algeria. Plant Ecology and Diversity, 16(1), 45–63.

22. Benmansour, H., & Gherbi, S. (2024). Mycological contamination profiles of topical formulations and compliance with North African regulatory standards. Maghrebian Journal of Pharmaceutical Sciences, 19(1), 45–58.

23. Benmansour, H., & Gherbi, S. (2024). Physicochemical emulsification failures and latent mycological deterioration in topical cosmetic systems over prolonged storage. Maghrebian Journal of Pharmaceutical Sciences, 19(3), 142–156.

24. Bergey, D.H., Holt, J.G., 2000. Bergey's manual of determinative bacteriology. 9th ed. Philadelphia: Lippincott Williams & Wilkins.

25. Bessaoud, O., Pellissier, J. P., Rolland, J. P., & Khechimi, W. (2022). Agriculture and food security in North Africa: Climate change impacts and adaptation strategies. Springer.

26. Bhosale, S., Jadhav, A., Jamadar, A., & Ghongade, A. (2024). Formulation & evaluation of herbal cream using extract of: Alpinia galanga L. Willd. International Journal of All Research Education and Scientific Methods, 12(4), 4738–4745.

27. Bouzid, M., et al. (2022). Vascular plant diversity and habitat specificity in the Saharan Atlas and pre- Saharan zones of Algeria. Journal of Plant Ecology, 15(4), 345–360.

28. CABI Compendium. (2023). *Leucaena leucocephala* datasheet. CAB International.

29. CABI Compendium. (2023). *Leucaena leucocephala* (leucaena) – Datasheet. CAB International.

30. Campbell, S., Vogler, W. D., Brazier, D., & Brooks, S. J. (2019). Weed leucaena and its significance, implications and control. In Proceedings of the International Leucaena Conference (pp. 14–22). Tropical Grasslands-Forrajes Tropicales.

31. Carvalho, R., Pedrosa, C., Leal, A., Palermo, L. C., & Mansur, C. (2021). Extraction, characterization and rheological behavior of galactomannans in high salinity and temperature conditions. *International Journal of Polymer Analysis and Characterization*, *26*(7), 573–592.

32. Casanova-Lugo, F., Petit-Aldana, J., Solorio-Sánchez, F. J., & Parsons, D. (2014). Forage yield and quality of *Leucaena leucocephala* and *Guazuma ulmifolia* in mixed and pure fodder banks systems in Yucatan, Mexico. *Agroforestry Systems*, *88*(1), 29–39.

33. Chaer, G. M., Resende, A. S., Campello, E. F. C., de Faria, S. M., & Boddey, R. M. (2011). Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. *Tree Physiology*, *31*(2), 139–149..

34. Cobzaru, C., Apostolescu, N., & Cernatescu, C. (2025). Formulation and Microbiological Evaluation of a Cosmetic Cream with Sea Buckthorn Extract and Without Preservatives. Zenodo.

35. Condalab. (2020). Microbiological analysis of cosmetics [Technical guide]. Condalab. https://www.chemie-brunschwig.ch/documents/suppliers-information/condalab/Microbiological-Analysis-Cosmetics_EN_2020.pdf

36. Cosmetic Ingredient Review Expert Panel. (2015). Safety assessment of galactomannans as used in cosmetics. International Journal of Toxicology, 34(1S), 5–19.

37. Da Silva N., et al. “Microbiological examination methods of food and water: A Laboratory Manual. CRC Press (2018)

38. De Sousa Machado, M. T., Drummond, J. A., & Barreto, C. (2020). *Leucaena leucocephala* (Lam.) de Wit in Brazil: History of an invasive plant. *Estudos Ibero-Americanos*, *46*(1), Article e33976.

39. Dholakia, P., Sabharwal, P. K., & Vandana. (2022). Seed gums: Sources, applications, and recent trends in edible films. In M. W. Iqbal & X. Qi (Eds.), *Edible food packaging: Applications, innovations and sustainability* (pp. 263–276).

40. Di Mambro, V. M., & Fonseca, M. J. V. (2005). Assays of physical stability and antioxidant activity of a topical formulation added with different plant extracts. Journal of Pharmaceutical and Biomedical Analysis, 37(2), 287–295

41. Dini, I., & Laneri, S. (2021). The new challenge of green cosmetics: Natural food ingredients for cosmetic formulations. Molecules, 26(13), Article 3921.

42. Dos Santos, V. R. F., Souza, B. W. S., Teixeira, J. A., Vicente, A. A., & Cerqueira, M. A. (2015). Relationship between galactomannan structure and physicochemical properties of films produced thereof. *Journal of Food Science and Technology*, *52*(12), 8292–8299.

43. Eid, A.- A., et al. (2025). Innovative properties of sustainable galactomannans from seeds of Adenanthera pavonina and their applications in cosmetics. ScienceDirect review on Progress in galactomannan- based materials for biomedical and cosmetic applications (2025).

44. Faluan, S., Intanon, M., Suriyasathaporn, W., & Awaiwanont, N. (2025). Effects of galacto-oligosaccharides on growth performance and gut health in broiler chicken. *Veterinary Integrative Sciences*, *23*(1), Article e2025029.

45. Flores García, Y., Martín del Campo Solís, M. F., Gómez-Angulo, J. H., Martínez Preciado, A. H., Silva-Jara, J. M., Padilla de la Rosa, J. D., & Escalante-Garcia, Z. Y. (2025). Industrial applications, principal sources, and extraction of galactomannans: A review. *Foods*, *14*(9), Article 1587.

46. Franceschini, M., Pizzetti, F., & Rossi, F. (2025). On the key role of polymeric rheology modifiers in emulsion-based cosmetics. Cosmetics, 12(2), 76.

47. Gabriela Ibieta , Atma-Sol Bustos , Jimena Ortiz-Sempértegui , Javier A Linares-Pastén , J Mauricio Peñarrieta. (2023). Molecular characterization of a galactomannan extracted from Tara (Caesalpinia spinosa).

García, Y. F., et al. (2024). Industrial Applications, Principal Sources, and Extraction of Galactomannans: A Review. Polymers and Bio-composites Journal, 16(3), 105-120.

48. García- Solís, P., et al. (2025). Industrial applications, principal sources, and extraction of galactomannans. International Journal of Biological Macromolecules, 257, 129871.

49. García- Solís, P., et al. (2025). Industrial applications, principal sources, and extraction of galactomannans. International Journal of Biological Macromolecules, 257, 129871.

50. Global Biodiversity Information Facility (GBIF). (2024). *Leucaena leucocephala* (Lam.) de Wit – Species distribution map.

51. Guiraud, J. P. (1998). *Microbiologie alimentaire*. Dunod, Paris, p. 696.

52. GuleriaA .,(2014). Isolation and Identification of Bacteria from Different Cosmetic Samples and to Check Antimicrobial Activity of Antibiotics on Bacteria Isolated. Int. J. Scientific Res. 3 (2), 462.

53. Halla, N., Fernandes, I. P., Heleno, S. A., Costa, P., Boucherit-Otmani, Z., Boucherit, K.,Rodrigues, A. E., Ferreira, I. C. F. R., & Barreiro, M. F. (2018). Cosmetics preservation: A review on present strategies. Molecules, 23(7), 1571.

54. Jairoun, A. A., Al-Hemyari, S. S., Shahwan, M., & Zyoud, S. H. (2020). An Investigation into Incidences of Microbial Contamination in Cosmeceuticals in the UAE: Imbalances between Preservation and Microbial Contamination. Cosmetics, 7(4), 92.

55. Ji, S., Balista, P. A., Dario, M., & Gonçalves, F. S. M. (2024). Evaluation of the stability and antioxidant activity of formulations with Mangifera indica L. extract. O Mundo da Saúde, 48, Article e15832024I.

56. Joice M. Scheibel , Nataly M. Siqueira , Laiane S. da Silva , Manoela A.M. Mace , Rosane M.D. Soares (2025). Progress in galactomannan- based materials for biomedical and cosmetic applications.

57. Kallings, L. O., Ringertz, O., & Silverstolpe, L. (1969). Microbiological contamination of medical preparations, cosmetics, and toilet articles. Acta Pharmaceutica Suecica, 6(4), 433–438.

58. Kekou, C., Yapi, Y. M., Koutouan, F. P., Tiho, T., & Kimse, M. (2020). Bioactive compounds and antioxidant activity of *Leucaena leucocephala* leaves collected in Côte d'Ivoire. *Journal of Applied Life Sciences International*, *23*(12), 48–56.

59. Kontogiorgos, V. (2018). Galactomannans (Guar, Locust Bean, Fenugreek, Tara).In *Reference Module in Food Science*. Elsevier.

60. Korać, R. R., & Khambholja, K. M. (2011). Potential of herbs in skin protection from ultraviolet radiation. Pharmacognosy Reviews, 5(10), 164–173.

61. Kumar, R., Sharma, V., & Singh, B. (2021). Galactomannans: Structural characteristics and applications in drug delivery and cosmetics. Carbohydrate Polymers, 256, 117589.

62. Legume Genome Consortium. (2023). Global diversity and genomic resources of the Fabaceae family. Trends in Plant Science, 28(4), 456–470.

63. Leyva-Mayorga, M., & Santos-Sánchez, N. F. (2018). Kinetic growth of yeasts in lipid-rich food matrices and emulsion stability. Food Microbiology, 72, 112-120

64. Li, Y., Wang, Q., & Zhang, X. (2023). Rheological behavior and molecular characteristics of leguminous galactomannans in aqueous dispersions. LWT – Food Science and Technology, 178, 114589.

65. Liu, J. K. (2022). Natural products in cosmetics. *Natural Products and Bioprospecting*, *12*(1), Article 40.

66. Liu, Y., Lei, F., He, L., Xu, W., & Jiang, J. (2020). Physicochemical characterization of galactomannans extracted from seeds of *Gleditsia sinensis* Lam and fenugreek. Comparison with commercial guar gum. *International Journal of Biological Macromolecules*, *158*, 1047–1054.

67. Marcilia P Costa , Maurycyo S Geronço, Igor F da S Ramos , Monalisa de A Lucena , Ricardo Araújo , Iluska M Pinheiro , Rayran Walter R de Sousa , Paulo Michel P Ferreira , Josy A Osajima , Manuela Amorin , Óscar Ramos , Edson C Silva-Filho , Alessandra B Ribeiro , Marcia Dos S Rizzo (2025). Innovative properties of sustainable galactomannans from seeds of Adenanthera pavonina and their applications in cosmetics. ScienceDirect review on Progress in galactomannanbased materials for biomedical and cosmetic applications (2025).

68. Martínez-García, L. M., et al. (2023). Root-nodule symbioses and nitrogen cycling in desert legumes. Plant, Cell & Environment, 46(5), 1567–1582.

69. McClements, D. J. (2015). Food emulsions: principles, practices, and techniques (3rd ed.). CRC Press.

70. Mittal, N., & Kaur, G. (2019). *Leucaena leucocephala* (Lam.) galactomannan nanoparticles: Optimization and characterization for ocular delivery in glaucoma treatment. International Journal of Biological Macromolecules, 139, 1252–1262.

71. Mittal, N., Mattu, P., & Kaur, G. (2016). Extraction and derivatization of *Leucaena leucocephala* (Lam.) galactomannan: Optimization and characterization. International Journal of Biological Macromolecules, 92, 831–841.

72. Mohamed Rashid Salama Rashid , Mohamed Ahmed Hanafy , Mervat Sayed Hassan Youssef , Harry Archimède , Sobhy Mohamed Abdallah Sallam , Yosra Ahmed Soltan , and Wafaa Mostafa Ali Ghoneem. (2021). Chemical and in vitro evaluation of Leucaena (*Leucaena leucocephala*) leaves as a substitute of alfalfa (Medicago sativa L.).

73. Monica, C., 2006. Microbiological tests: district laboratory practice in tropical countries. Press Syndicate of the University of Cambridge., 2(2): 35-70.

74. Monsigny, M., Petit, C., & Roche, A. C. (1988). Colorimetric determination of neutral sugars by a resorcinol-sulfuric acid micromethod. *Analytical Biochemistry*, 175(2), 525-530.

75. Naskar, A., & Kim, K. S. (2020). Recent advances in nanomaterial-based wound-healing therapeutics. *Pharmaceutics*, *12*(6), Article 499.

76. Neza, E., & Centini, M**.** (2015). Microbiological contamination of cosmetic products throughout their timeline of use: a review. *Journal of Cosmetics, Dermatological Sciences and Applications*, 5(04), 314.

77. Norouz-zadeh, S., Saeedi, M., Enayatifard, R., Morteza-Semnani, K., & Akbari, J. (2014). Microbial Content in some Foundation Creams in Iran's Market. Journal of Mazandaran University of Medical Sciences, 24(118), 214–219.

78. Nwokocha, L. M., Williams, P. A., & Gidley, M. J. (2022). Functional properties of galactomannans and their applications in food and pharmaceutical industries. Food Hydrocolloids, 124, 107251.

79. Odendo, M., Bationo, A., & Kimani, S. (2011). Socio-economic contribution of legumes to livelihoods in Sub-Saharan Africa. In A. Bationo (Ed.), Fighting poverty in Sub-Saharan Africa: The multiple roles of legumes in integrated soil fertility management (pp. 27–46). Springer.

80. Official Journal of the People's Democratic Republic of Algeria. (2020). Interministerial decree of 21 October 2019 establishing the microbiological criteria for cosmetic and body hygiene products. Journal Officiel de la République Algérienne Démocratique et Populaire, No. 16, March 24, 2020, 25–29

81. Official Journal of the People's Democratic Republic of Algeria. (2020). Interministerial Order of 21 October 2019 establishing the technical regulation defining the microbiological criteria for cosmetic and personal hygiene products. Official Journal of the People's Democratic Republic of Algeria, No. 16, March 24, 2020, pp. 25–29

82. Ogunniyi, T. A., et al. (2021). *Leucaena leucocephala*: Novel mutants with new chemical profiles. Egyptian Journal of Chemistry.

83. Padma Sharma , Amarpreet Kaur , Daizy R. Batish, Shalinder Kaur and Bhagirath S. Chauhan, et al. (2022). Critical insights into the ecological and invasive attributes of *Leucaena leucocephala*, a tropical agroforestry species. Frontiers in Agronomy, 2:890992.

84. Pandey, S., Akanksha, S., Rajesh, T., Sunita, S., Mishra, A., & Singh, N. (2014). Development and evaluation of antimicrobial herbal cosmetic preparation. *African Journal of Pharmacy and Pharmacology*, *8*(20), 514–528.

85. Pittet, A. (1998). Natural occurrence of mycotoxins in foods and feeds — an updated review. Revue de Médecine Vétérinaire, 149(6), 479-492.

86. Ramos, F. L., & Santos, C. M. (2025). Identifying airborne fungal contaminants and experimental artifacts in routine cleanroom microbiological monitoring. International Journal of Environmental Microbiology, 51(3), 204–218.

87. Ramos, F. L., Santos, C. M., & Gomez, M. A. (2025). Enzymatic breakdown of semi-solid organic matrices driven by ubiquitous Aspergillus and Penicillium strains. International Journal of Environmental Microbiology, 52(1), 88–103.

88. Ramos, F. L., Santos, C. M., & Gomez, M. A. (2025). Morphological tracking of melanized airborne molds and fungal spore germination in non-sterile compounding environments. International Journal of Environmental Microbiology, 51(4), 310–324

89. Reis Mansur, C. R., Leitão, S. G., & Santos, E. P. (2016). In vitro and in vivo evaluation of sun protection factor (SPF) of cosmetic formulations containing plant extracts. *International Journal of Cosmetic Science*, 38(6), 551-558.

90. Ribeiro, A. S., Estanqueiro, M., Oliveira, M. B., & Sousa Lobo, J. M. (2015). Main benefits and applicability of plant extracts in skin care products. *Cosmetics*, *2*(2), 48–65

91. Sagi, R., Devanaboyina, N., Nagireddy, N. K., & Boini, S. (2025). Nutritional characterisation of *Leucaena leucocephala* leaves: Insights for sustainable ruminant production. *Journal of Experimental and Applied Innovation*, *3*(2), 114–125.

92. Saleem, M., Ain, N. ul., Iqbal, M.N., 2020. Efficacy of Cleaning Agents against Bacterial Isolates from Raw Meat Sold in Market Places in Lahore. PSM Vet. Res., 5(1): 6–15.

93. Sciarini, L. S., Maldonado, F., Ribotta, P. D., Perez, G. T., & León, A. E. (2009). Chemical composition and functional properties of Gleditsia triacanthos gum. Food Hydrocolloids, 23(2), 306–313.

94. Senthil Raja, M. (2025). Formulation and evaluation of herbal cream extract for anti-acne activity. *International Journal of Research in Pharmaceutical Sciences*, *16*(1), 88–95.

95. Sharma, N., Bashir, O., Pawase, P. A., Gaur, S. S., & Amin, T. (2025). Structural, functional characteristics and diverse applications of galactomannans: A comprehensive review. Carbohydrate Research, 556, Article 109627

96. Sharma, P., Kaur, A., Batish, D., & Kaur, S. (2022). Critical insights into the ecological and invasive attributes of *Leucaena leucocephala*, a tropical agroforestry species. *Frontiers in Agronomy*, *4*, Article 890992.

97. Shirajuddin, S., Kamarun, D., Ismail, N. E., Abdul Wahab, M. S., Rosdi, M. N., & Che-Mohamad, N. A. (2015). Extraction and characterization of galactomannan from seeds of *Leucaena leucocephala*. *Advanced Materials Research*, *1134*, 213–219.

98. Spencer, J. F. T. and A. L. Spencer (2001). Food Microbiology Protocols. Humana Press. Totowa, New Jersey.

99. Ssenku, J. E., Ntale, M., Backeus, I., & Oryem-Origa, H. (2017). Phytoremediation potential of Leucaena leucocephala (Lam.) de Wit. for heavy metal-polluted and heavy metal-degraded environments. In A. A. Ansari, S. S. Gill, R. Gill, G. R. Lanza, & L. Newman (Eds.),Phytoremediation potential of bioenergy plants (pp. 189–209). Springer.

100. Structural, functional characteristics and diverse applications of galactomannans. Carbohydrate Polymers, 305, 120611. Nikhil Sharma 1, Omar Bashir 2, Prashant Anil Pawase 3, Supriya Singh Gaur 1, Tawheed Amin 4 (2025) . Affiliations+Expand

101. Sukamdi, D. P., Dewinda, Z. S., Damarwati, V. L., Maziyyah, N., & Ningrum, D. W. C. (2024). Evaluation of physical and chemical stability of semi-solid preparations towards beyond- use date. Acta Pharmaciae Indonesia, 11(2), 9260.

102. Tao, Y., et al. (2021). Hydrolyzed galactomannan from Trichoderma reesei: antioxidant and immunomodulatory activities. Carbohydrate Polymers, 272, 118512.

103. Techaoei, S. (2017). Bacterial and Fungal Contamination of Personal Care Product in Northern Thailand. Journal of Applied Research on Science and Technology, 16(1-2), 32–38.

104. Tedjani, A., Mouane, A., Azzi, M., & Boual, Z. (2025). Galactomannans from chemical insights to multifaceted applications: A review. *Natural Product Communications*, *20*(6), Article 1934578X251355362..

105. Tedjani, A., Mouane, A., Azzi, M., Boual, Z., Atoki, A. V., & Messaoudi, M. (2025). Galactomannans from chemical insights to multifaceted applications: A review. Natural Product Communications, 20(6), 1–12.

106. The Legume Phylogeny Working Group (LPWG). (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon*, *66*(1), 44–77

107. Trees and Shrubs Online. (2023). *Leucaena leucocephala*. Royal Horticultural Society (Trees and Shrubs Online).

108. Tsonkova, P., et al. (2024). Multipurpose leguminous trees for sustainable silvopastoral systems in the tropics. Land Degradation & Development, 35(3), 789–804.

109. UNESCO- MAB. (2021). Sustainable management of arid and semi- arid ecosystems in North Africa: Challenges and opportunities. UNESCO- MAB Technical Report No. 58.

110. Urooj, S., Narejo, A.H., Sahar, N. ul, Iqbal, M.N., 2022. Accessing the Fecal Pollution of the Aquifer Systems using Portable Microbiology Technique: An Affordable Approach to Enhance Coverage of Safely Managed Water Services. PSM Microbiol., 7(3): 73–81.

111. Wang, L., et al. (2017). Comparative analysis of polyphenol profiles and antioxidant activities in leaves versus seeds of Fabaceae family plants. Phytochemistry Analysis, 28(4), 310-318.

112. Wang, T., et al. (2021). Synthesis and characterization of new quaternized galactomannan derivatives for hair- care applications. International Journal of Biological Macromolecules.

113. Widad, M., Bouallala, M., Bradai, L., & Souddi, M. (2024). Floristic diversity of plant communities in sandy wadis of the northern Algerian Sahara (Ghardaïa region). Biodiversity Research and Conservation, 72, 1–10.

114. Wu, Y., Ren, A., Lv, X., Ran, T., Zhang, G., Zhou, C., & Tan, Z. (2022). Effects of galactomannan oligosaccharides on growth performance, mycotoxin detoxification, serum biochemistry, and hematology of goats fed mycotoxins-contaminated diets. *Frontiers in Veterinary Science*, *9*, Article 852251..

115. Yaquelin Flores García , Martha Fabiola Martín del Campo Solís , Jorge H Gómez-Angulo , Alma Hortensia Martínez Preciado , Jorge Manuel Silva-Jara , José Daniel Padilla de la Rosa , Zazil Y Escalante-Garcia. (2025). Industrial applications, principal sources, and extraction of galactomannans.

116. Zhang, L., et al. (2024). Structural and physicochemical characterization of plant- derived galactomannans: Revealing promising substrates for mannooligosaccharide production. Carbohydrate Polymers, 326, 121526.

117. Zhang, L., et al. (2024). Structural and physicochemical characterization of plant- derived galactomannans: Revealing promising substrates for mannooligosaccharide production. Carbohydrate Polymers, 326, 121526.

118. Zhang, Y., et al. (2023). Biologically active secondary metabolites from Fabaceae species: From structure to bioactivity. Phytochemistry Reviews, 22, 1121–1150.

119. Zhang, Y., et al. (2023). Recent advances on application of polysaccharides in cosmetics.

120. Zhao, M., et al. (2023). Role of plant- based galactomannans as film- forming and suspending agents in sunscreen and makeup formulations. International Journal of Cosmetic Science, 45(3), 234–247.

Annexes

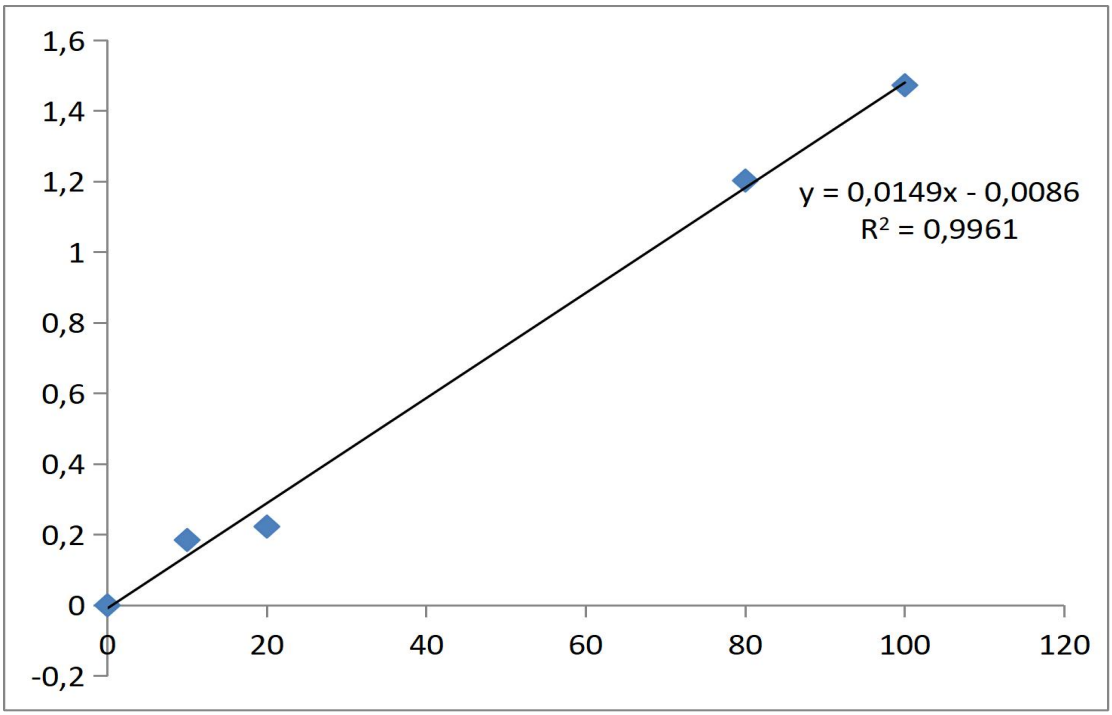


Figure27 : Calibration Curve for Neutral Sugars

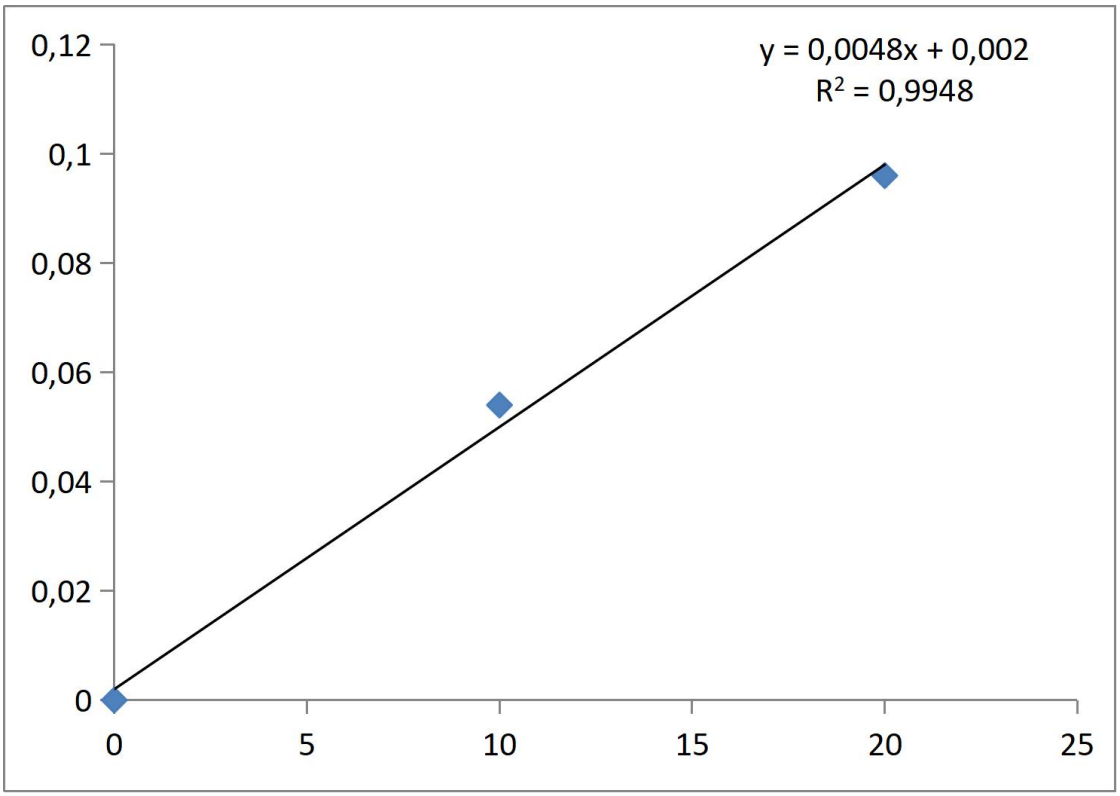


Figure28: Calibration Curve for protein

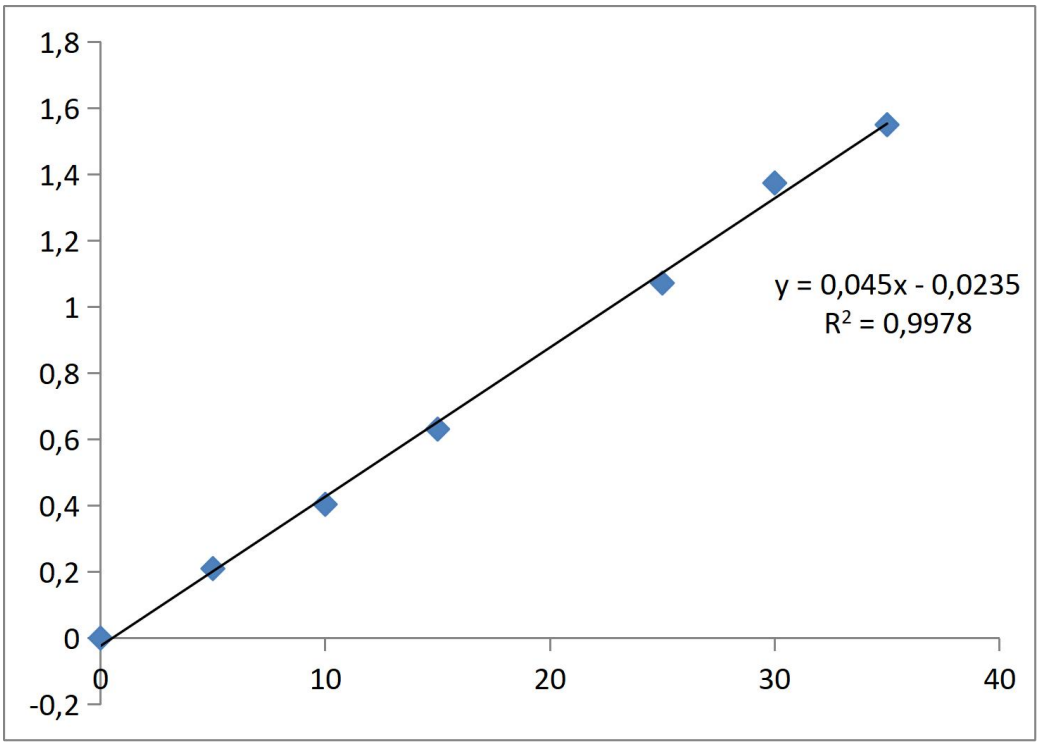


Figure 29 : Calibration Curve for total phénolique

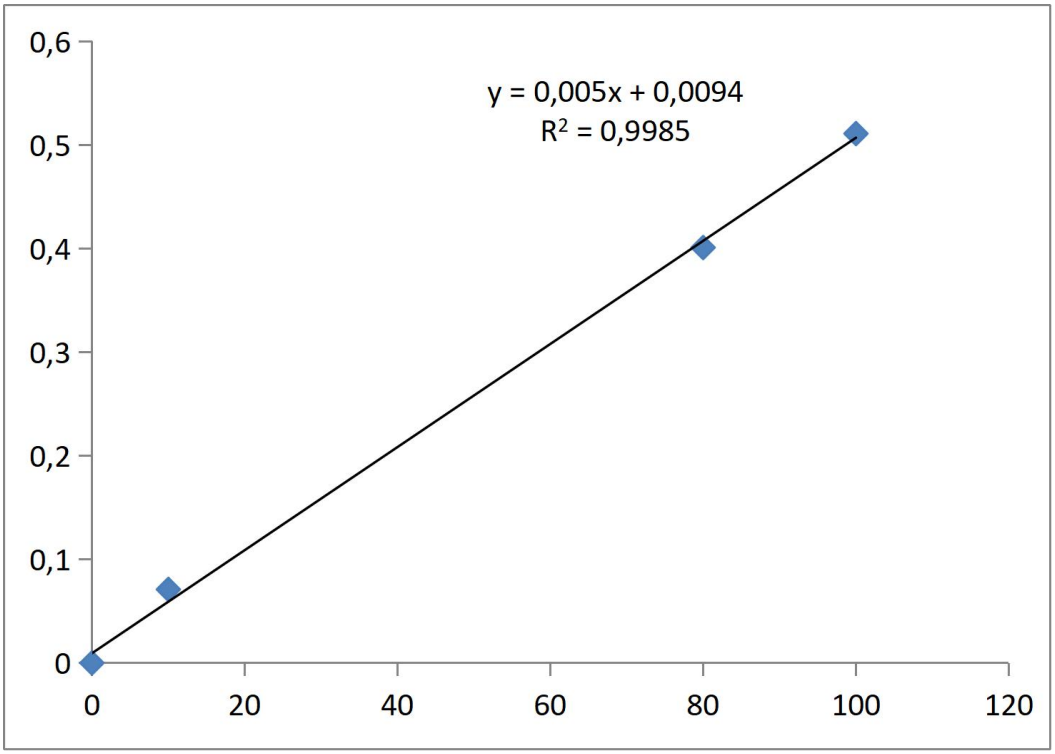


Figure30 : Calibration Curve for total sugar

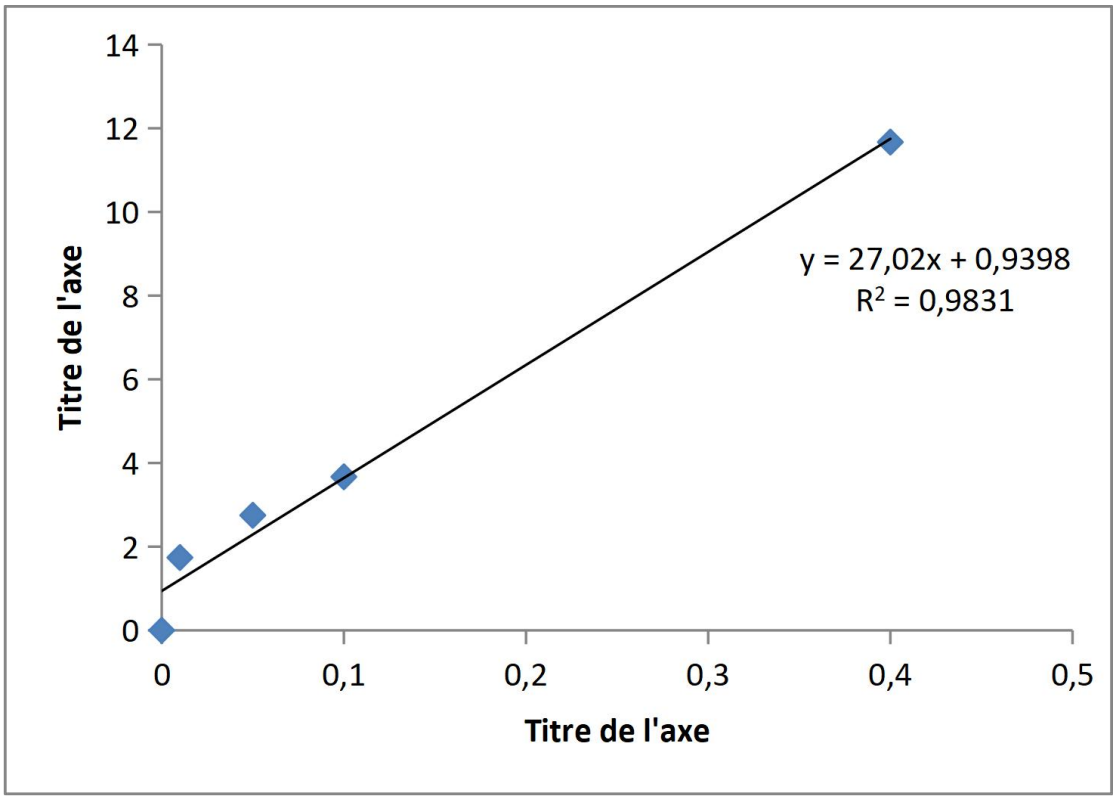


Figure31 : Calibration Curve of Cream for the DPPH

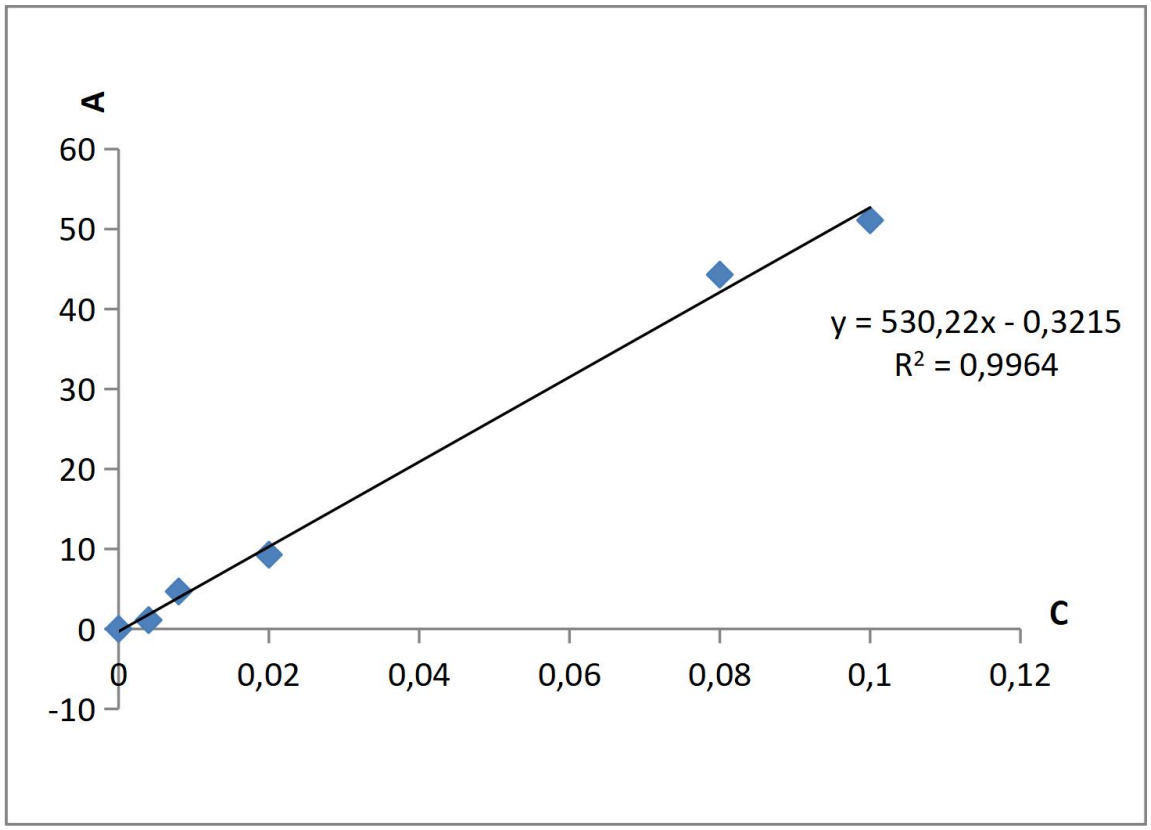
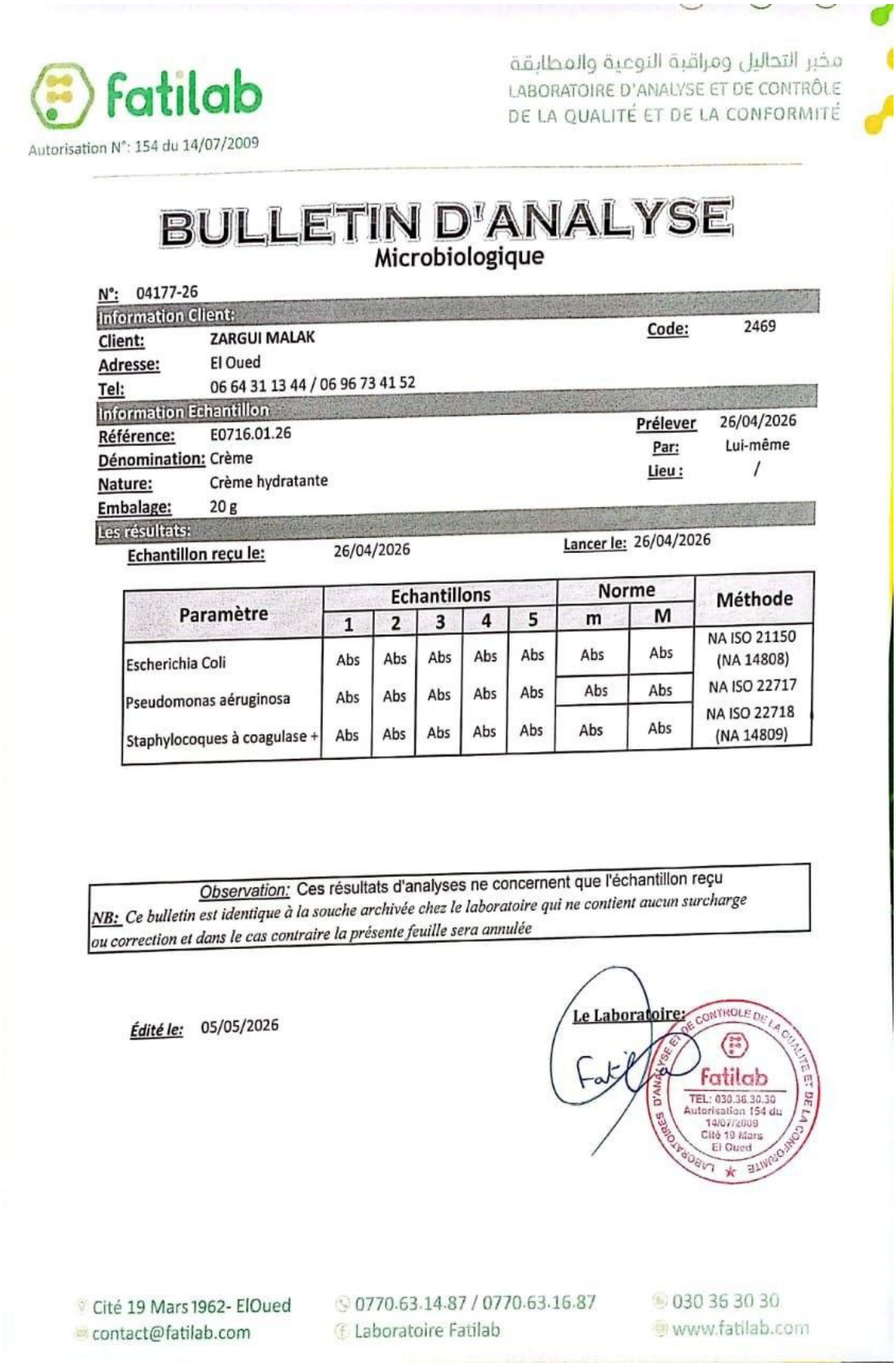


Figure32 : Calibration Curve of ascorbic acid for the DPPH



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Autorisation N°: 154 du 14/07/2009

مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE
DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE
Microbiologique

N°: 04177-26

Information Client:

Client: ZARGUI MALAK — **Code:** 2469
Adresse: El Oued
Tel: 06 64 31 13 44 / 06 96 73 41 52

Information Echantillon

Référence: E0716.01.26 — **Prélever** 26/04/2026
Dénomination: Crème — **Par:** Lui-même
Nature: Crème hydratante — **Lieu :** /
Embalage: 20 g

Les résultats:

Echantillon reçu le: 26/04/2026 — **Lancer le:** 26/04/2026

Paramètre	Echantillons					Norme		Méthode
	1	2	3	4	5	m	M	
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 21150 (NA 14808)
Pseudomonas aéruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22717
Staphylocoques à coagulase +	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22718 (NA 14809)

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu

NB: Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée

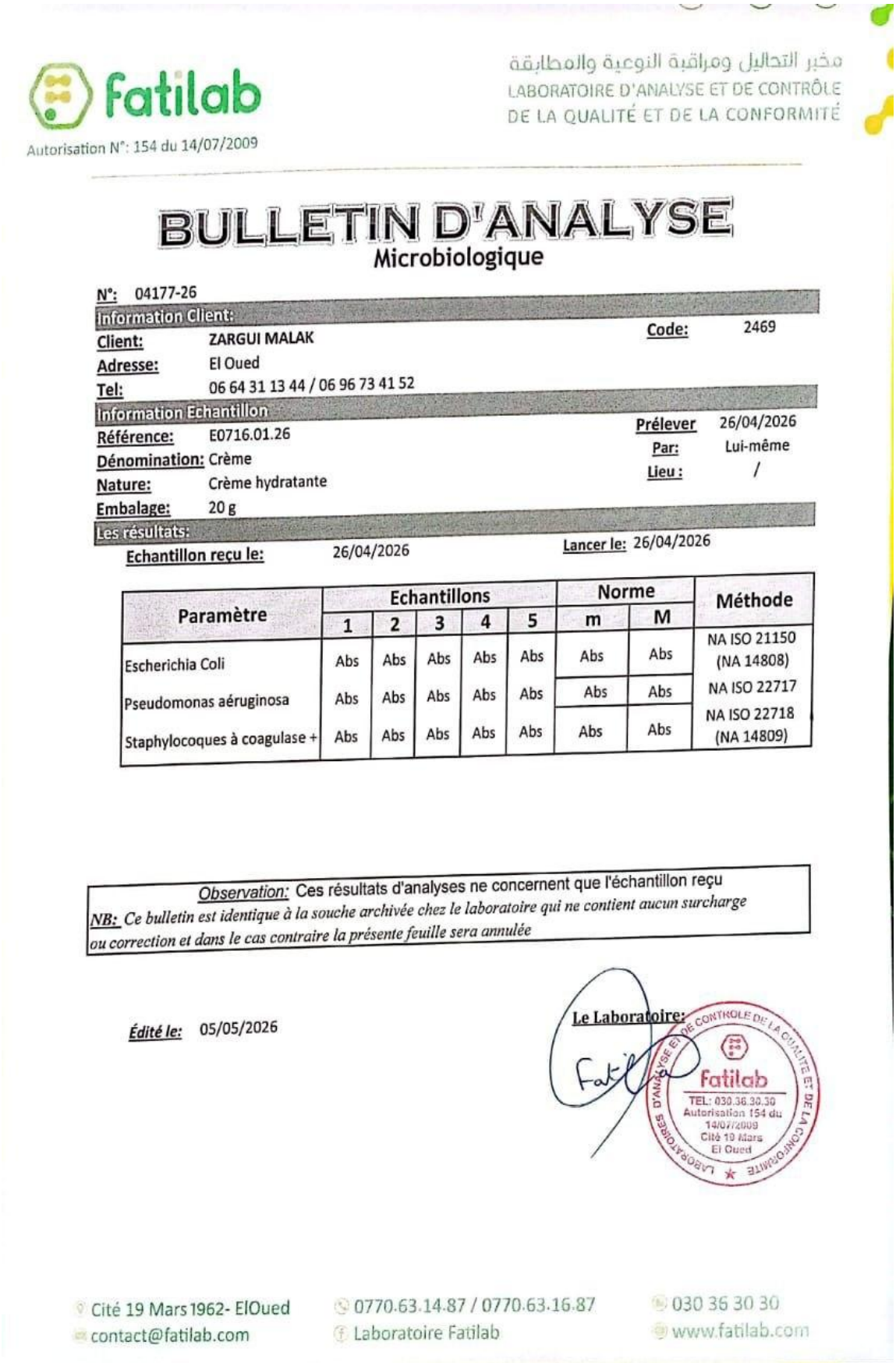
Édité le: 05/05/2026

Le Laboratoire:

LABORATOIRES D'ANALYSE ET DE CONTROLE DE LA QUALITE ET DE LA CONFORMITE
Fatilab
TEL: 030.36.30.30
Autorisation 154 du 14/07/2009
Cité 19 Mars El Oued

Cité 19 Mars 1962- ElOued
contact@fatilab.com
0770.63.14.87 / 0770.63.16.87
Laboratoire Fatilab
030 36 30 30
www.fatilab.com

Figure 33: Detection of the pathogen bacteria after 7 day



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Autorisation N°: 154 du 14/07/2009

مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE
DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE
Microbiologique

N°: 04177-26

Information Client:

Client: ZARGUI MALAK — **Code:** 2469
Adresse: El Oued
Tel: 06 64 31 13 44 / 06 96 73 41 52

Information Echantillon

Référence: E0716.01.26 — **Prélever** 26/04/2026
Dénomination: Crème — **Par:** Lui-même
Nature: Crème hydratante — **Lieu :** /
Embalage: 20 g

Les résultats:

Echantillon reçu le: 26/04/2026 — **Lancer le:** 26/04/2026

Paramètre	Echantillons					Norme		Méthode
	1	2	3	4	5	m	M	
Escherichia Coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 21150 (NA 14808)
Pseudomonas aéruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22717
Staphylocoques à coagulase +	Abs	Abs	Abs	Abs	Abs	Abs	Abs	NA ISO 22718 (NA 14809)

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu

NB: Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée

Édité le: 05/05/2026

Le Laboratoire:

LABORATOIRE D'ANALYSE ET DE CONTROLE DE LA QUALITE ET DE LA CONFORMITE
Fatilab
TEL: 030.36.30.30
Autorisation 154 du 14/07/2009
Cité 19 Mars El Oued

Cité 19 Mars 1962- ElOued — 0770.63.14.87 / 0770.63.16.87 — 030 36 30 30
contact@fatilab.com — Laboratoire Fatilab — www.fatilab.com

Figure 34 : Detection of the pathogen bacteria after 14 day

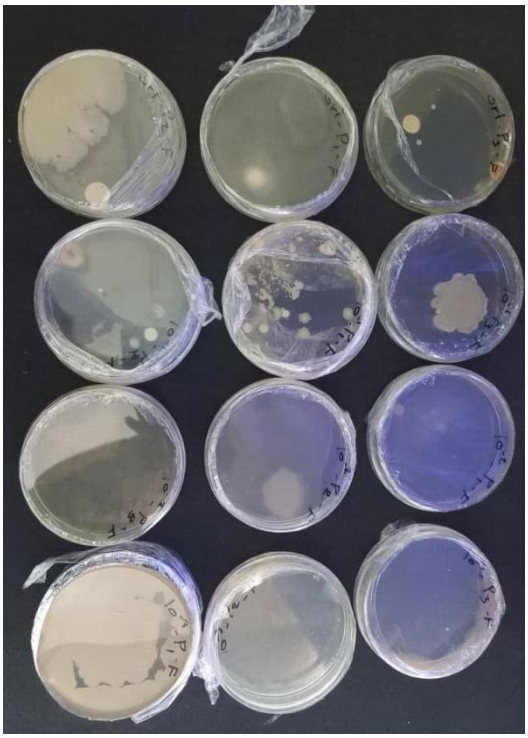

Figure 35: Fungal count and colony morphology after 7days of Cream Preparation

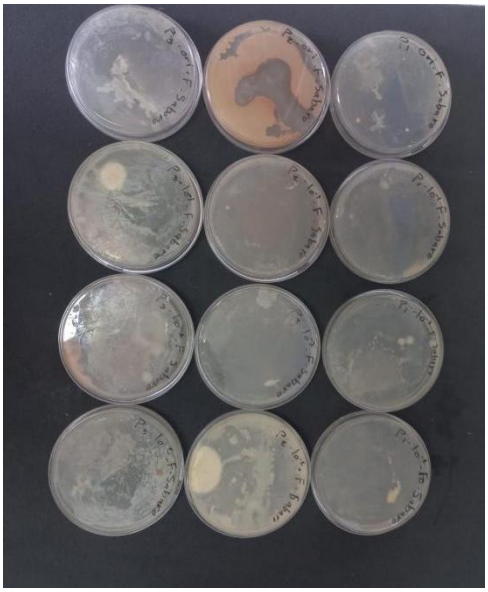

Figure 36: Fungal count and colony morphology after 16 days of Cream Preparation

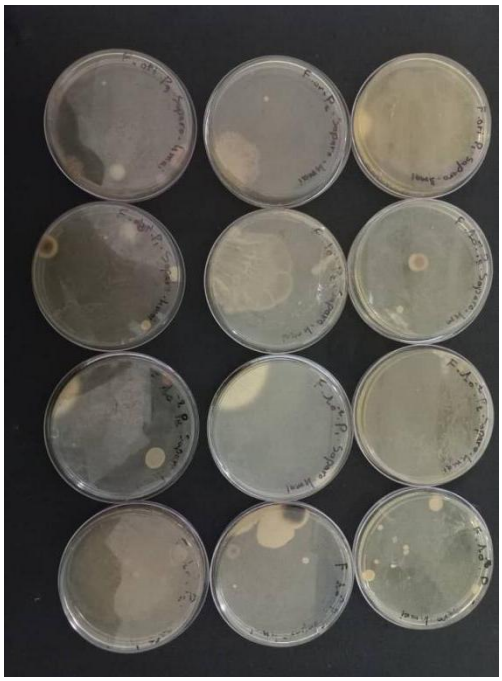

Figure 37: Fungal count and colony morphology after 21 days of Cream Preparation

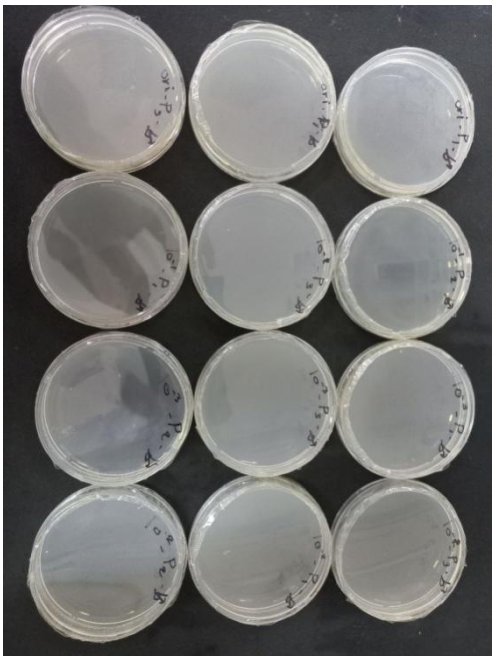

Figure 38 : Bacterial count and colony morphology after 24 hours of Cream Preparation

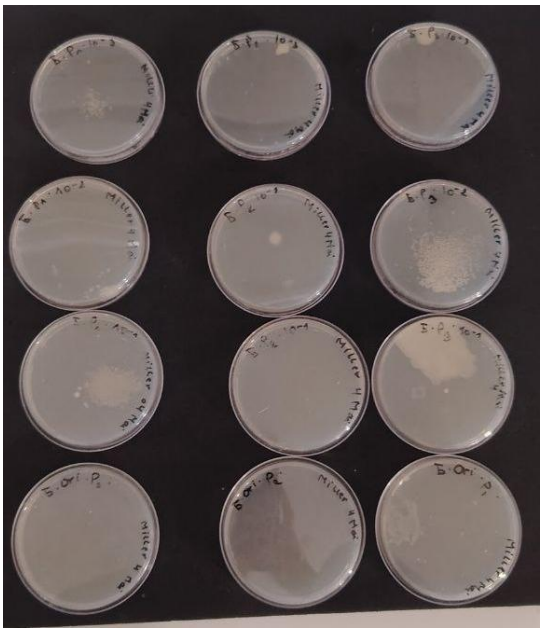

Figure 39 : Bacterial count and colony morphology after 21 days of Cream Preparation

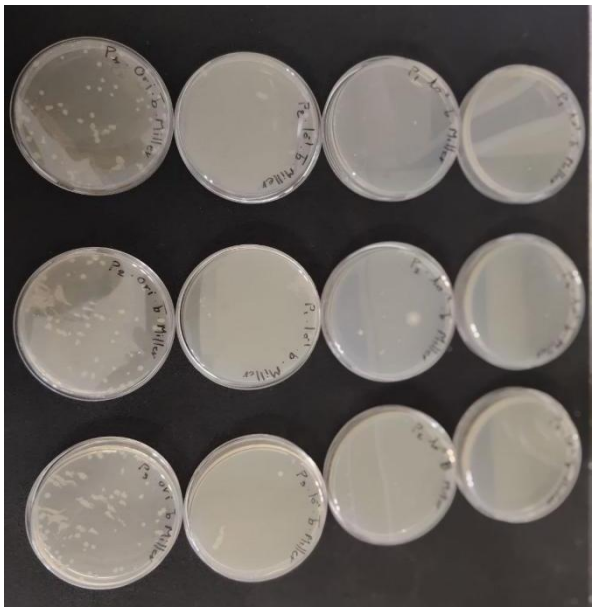

Figure 40 : Bacterial count and colony morphology after 7 days of Cream Preparation

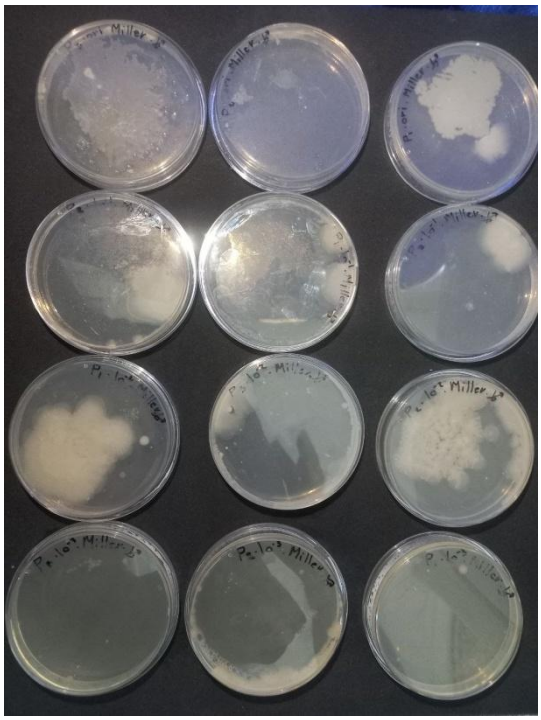

Figure 41 : Bacterial count and colony morphology after 14 days of Cream Preparation

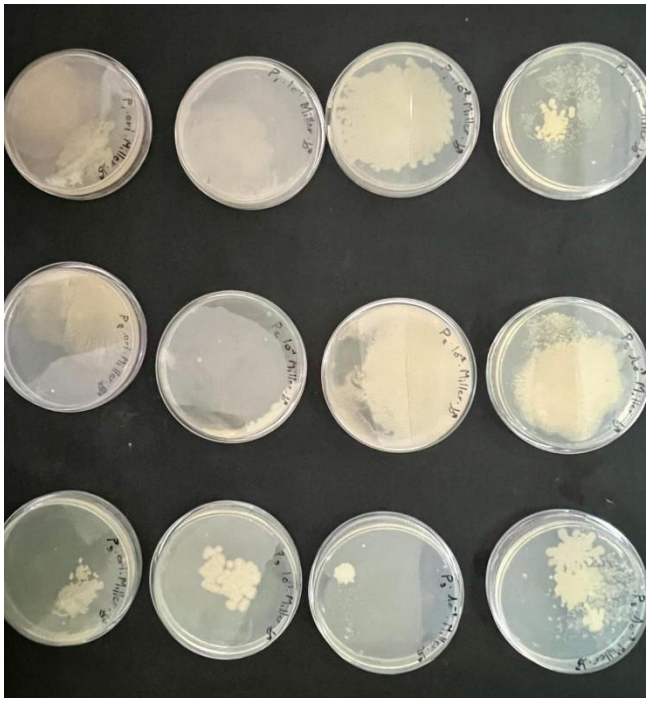

Figure 42 : Bacterial count and colony morphology after 16 days of Cream Preparation

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