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Speciality : Toxicology

THEME

Physico-chemical characterization and application of the water-soluble polysaccharides extracted from *Retama raetam* seeds

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Thanks

First and foremost, praise be to Allah, the Almighty, who said in His noble book "لئن شكرتم لأزيدنكم" All praise is due to Allah, out of love, gratitude, and appreciation, at the beginning and the end (وآخر دعواهم ان الحمدلله رب العالمين)

All praise is due to Allah who has guided us, for we would not have been guided if it were not for Allah's guidance. And may peace and blessings be upon " سيدنا محمد صلى الله عليه وسلم " , and upon his pure family and companions. As we conclude this scientific effort, we cannot but express our sincere gratitude and appreciation to all those who have assisted and supported us in completing this research.

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Dedication

In the name of Allah, the Most Gracious, the Most Merciful. All praise is due to Allah, who guided us and elevated us, and we could not have succeeded or excelled except by His satisfaction. All praise is due to Allah, who guided us through paths and efforts, none of which we could have traversed or exceeded except by His grace, to whom belongs all virtue, perfection, and completeness.

To the leader of the mindful, the master of the early and the latter, the teacher of teachers, and the messenger of his Lord to the worlds "سيدنا محمد صلى الله عليه وسلم"

To those whose satisfaction was a call in life, to those whose prayers were a cause of our success, to those who awaited this moment eagerly, to our beloved parents to those who sacrificed everything dear for our happiness and education, to the light that never fades in our hearts.

We dedicate this success first to ourselves, then to all who strove with us to complete this journey. You remain a constant support for us, and to our dear friend ***Hadil Haddadj****, whom we wish to experience the same joy of success next year and we pray for her a successful fortune, God willing.*

The journey was not short, and the matters were not easy, but with the help of Allah, we did it, and all praise is due to Allah. And the last of their call is,

"Ghoul Inas; Mazouzi Roumaissa ; Necib Asma; Ouaar Hadjer"

"وآخر دعواهم ان الحمد لله رب العالمين."

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿وَأَن لَّيْسَ لِلْإِنسَٰنِ إِلَّا مَا سَعَىٰ

وَأَنَّ سَعْيَهُۥ سَوْفَ يُرَىٰ﴾

صَدَقَ اللهُ العَظيمُ

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%	Percentage
°C	Degrees Celsius
A.Gal	D-galacturonic acid
A.Glc	D-glucuronic acid
AA	Ascorbic acid
ABTS	2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
AOAC	Association of Analytical Communities.
APX	Ascorbate peroxidase Bovine
B1	Biofilm concentration 1%
B1.5	Biofilm concentration 1.5%
BSA	Bovine Serum Albumin
CAT	catalase
CWSP	Cell wall storage polysaccharides
DO	Optical Density
DPPH	2,2-diphenyl 1-1picrylhydrazyl
XRD	X-ray diffraction
DNTB	5,5'-dithiobis-(2-nitrobenzoic acid)
FDA	Food and Drug Administration
FT-IR	infrared spectroscopy
FAT	Generally Recognized As Safe
g	Gram
Gal	D-galactose

GAL	Galactokinase
GALT	Galactose-1-phosphate uridylyltransferase
GGT	Galactomannan galactosyl transferase
GK	Glucokinase
Glc-6-P	Glucose-6-phosphate
Glue	D-glucose
GM	Galactomannan
h	Hour
H2SO4	Sulfuric acid
IC50	Concentration inhibition 50% just
JAR	Just about-right
KI	Potassium iodide
LBG	Locust Bean Gum
M	Molar
M/G	Mannose/Galactose
Man	D-mannose
mg	Milligram
min	Minutes
mm	Millimeter
Na2S2O3	sodium thiosulfate
nm	Nanometer
PH	Potential hydrogen
POD	Peroxidase
PSRR	Seed polysaccharides by Retama Raetam

PV	peroxide value
ROS	Reactive oxygen species
RSA	Radical scavenging activity
RVLL	Regional Veterinary Laboratory of Laghouat
S	Seconds
SEM	Scanning electron microscope
SH	Sulfhydryl group
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substance
TrisEDTA	Ethylene diamine tetraacetic
TS	Too strong
TW	Too weak
V	Volume
VOCs	volatile organic compounds
µL	Micro liter

Abstract

This study aimed to extract and to characterize the water-soluble polysaccharides from *Retama raetam* seeds harvested from the El Oued region and to apply it as edible biopolymer for food packaging. The sugar was extracted by hot water maceration followed by ethanol precipitation, Total sugars, neutral sugars, polyphenols, and proteins contenants were mesured. Structural properties of the extract were studied using UV-Visible, FTIR, XDR , and SEM-EDX technic. Biofilms was prepared at different concentrations, and thickness, opacity, moisture content, water contact angle, and solubility were measured. Then, the biofilm was applied in cheese packaging. Results showed an extraction yield equal to 7%. Chemical analysis of the raw sugar extract revealed a total sugar concentration of was 70%. The neutral sugar content was approximately 54,60%. Protein content was 6,27%. Polyphenol content was 1,7%. Structural characterization confirmed that the polysaccharides is of galactomannans type. Biofilms were prepared at two concentrations (1% and 1,5%) thickness measurements were 0,195 mm and 0,226 mm, respectively. While opacity were 0,303% and 0,024%, respectively. However, moisture content were 20% and 14%, respectively. Both biofilms exhibited 100% solubility in Water. Either the contact angle with water was 0° for the two biofilms. The Tests conducted on cheese demonstrated the effectiveness of the biopolymer biofilms in preserving the integrity in terms of gloss, color, appearance, and odor under different conditions (25°C and 4°C), as indicated by pH measurements, cheese weight, and protein and fat oxidation tests which showed weak oxidation. Regarding the biological activity of the biofilms, the result showed an antioxidant activity by the inhibition of the DPPH radical with an IC_{50} values of 0,40 Mg/ml and 0,46 Mg/ml for the 1% and 1,5% biofilm respectively. Either, the potential antibactrial activity against two bacterial strains was observed at different concentrations, resulting in inhibition zones ranging from 0,40 mm to 2,06 mm for *Salmonella enterica* and *E.coli*. In conclusion, this study developed that the obtained biofilms have potential applications in food packaging, due to their ability to preserve food integrity and inhibit bacterial growth.

Key words: *Retama raetam,* galactomannan, biofilms, cheeze packaging, antibactrial activity, antioxidant activity.

ملخص

هدفت هذه الدراسة إلى استخلاص وتوصيف السكريات المتعددة القابلة للذوبان في الماء من بذور نبات *Retama raetam* التي تم حصادها من منطقة الوادي، وتطبيقها كبوليمر صالح للأكل لتغليف الأغذية. تم استخلاص السكر عن طريق النقع في الماء الساخن ثم الترسيب بالإيثانول. تم قياس محتويات السكريات الكلية والسكريات المحايدة والبوليفينولات والبروتينات. تم دراسة الخصائص الهيكلية للمستخلص باستخدام تقنيات UV-Visible و FTIR و XDR و SEM-EDX. تم تحضير الأغشية الحيوية بتراكيزمختلفة، وتم قياس السماكة، والعتامة، ومحتوى الرطوبة، وزاوية التماس مع الماء، والذوبانية. ثم تم تطبيق الغشاء الحيوي في تغليف الجبن. أظهرت النتائج أن مردودية الاستخلاص تساوي 7%. وكشفت التحليلات الكيميائية لمستخلص السكر الخام عن تركيز سكريات كلية قدره 70%. كان محتوى السكريات المحايدة حوالي 54.60%. كان محتوى البروتين 6.27%. بينما كان محتوى البوليفينول 1.7%. وأكد التوصيف الهيكلي أن السكريات المتعددة من نوع الجالاكتومنان. تم تحضير الأغشية الحيوية بتركيزين (1% و1.5%) وكانت قياسات السماكة 0.195 مم و0.226 مم على التوالي. بينما كانت العتامة 0.303% و0.024% على التوالي. أما محتوى الرطوبة فكان 20% و14% على التوالي. أظهرت كلا الأغشية الحيوية قابلية ذوبان في الماء بنسبة 100%. وكانت زاوية التماس مع الماء 0° لكلا الأغشية. أظهرت الاختبارات التي أجريت على الجبن فعالية الأغشية الحيوية البوليمرية في الحفاظ على السلامة من حيث اللمعان، اللون، المظهر، والرائحة تحت ظروف مختلفة (C°25 وC°4)، كما أظهرت قياسات درجة الحموضة ووزن الجبن واختبارات أكسدة البروتين والدهون ضعف الأكسدة. فيما يتعلق بالنشاط البيولوجي للأغشية الحيوية، أظهرت النتائج نشاطًا مضادًا للأكسدة من خلال تثبيط جذر DPPH بقيم IC50 بلغت 0.40 Mg/ml و0.46 Mg/ml للغشاء الحيوي بنسبة 1% و1.5% على التوالي. كما لوحظ النشاط المضاد للبكتيريا ضد سلالتين بكتيريتين عند تركيزات مختلفة، حيث تتراوح مناطق التثبيط من 0.40 مم إلى 2.06 مم. *Salmonella enterica* و*E.coli*. في الختام، أثبتت هذه الدراسة أن الأغشية الحيوية المستخرجة لديها تطبيقات محتملة في تغليف الأغذية، نظرًا لقدرتها على الحفاظ على سلامة الأغذية ومنع نمو البكتيريا.

الكلمات المفتاحية: *Retama raetam* ، جلاكتومانان، أغشية حيوية، تغليف الجبن ، نشاط مضاد للبكتيريا ، نشاط مضاد للأكسدة .

Résumé

Cette étude visait à extraire et à caractériser les polysaccharides hydrosolubles des graines de *Retama raetam* récoltées dans la région d'El Oued et à les appliquer comme biopolymère comestible pour l'emballage alimentaire. Le polysaccharides a été extrait par macération à l'eau chaude suivie d'une précipitation à l'éthanol. Les contenus en oses totaux, oses neutres, polyphénols et protéines ont été mesurés. Les propriétés structurales de l'extrait ont été étudiées en utilisant les techniques UV-Visible, FTIR, XDR et SEM-EDX. Des biofilms ont été préparés à différentes concentrations, et l'épaisseur, l'opacité, la teneur en humidité, l'angle de contact avec l'eau et la solubilité ont été mesurés. Ensuite, le biofilm a été appliqué pour l'emballage du fromage. Les résultats ont montré un rendement d'extraction égal à 7 %. L'analyse chimique de l'extrait brut de sucre a révélé une concentration totale en sucre de 70 %. La teneur en sucres neutres était d'environ 54,60 %. La teneur en protéines était de 6,27 %. La teneur en polyphénols était de 1,7 %. La caractérisation structurale a confirmé que les polysaccharides est de type galactomannanes. Les biofilms ont été préparés à deux concentrations (1 % et 1,5 %) et les mesures d'épaisseur étaient de 0,195 mm et 0,226 mm, respectivement. Alors que l'opacité était de 0,303 % et 0,024 %, respectivement. Cependant, la teneur en humidité était de 20 % et 14 %, respectivement. Les deux biofilms ont montré une solubilité à 100 % dans l'eau. De plus, l'angle de contact avec l'eau était de 0° pour les deux biofilms. Les tests effectués sur le fromage ont démontré l'efficacité des biofilms biopolymères à préserver l'intégrité en termes de brillance, couleur, apparence et odeur dans différentes conditions (25°C et 4°C), comme indiqué par les mesures de pH, pert du poids du fromage et les tests d'oxydation des protéines et des graisses, qui ont montré une faible oxydation. En ce qui concerne l'activité biologique des biofilms, les résultats ont montré une activité antioxydante par l'inhibition du radical DPPH avec des valeurs IC50 de 0,40 Mg/ml 0,46 Mg/ml pour les biofilms à 1 % et 1,5 %, respectivement. De plus, une activité antibactérienne potentielle contre deux souches bactériennes a été observée à différentes concentrations, résultant en des zones d'inhibition allant de 0,40 mm à 2,06 mm pour *Salmonella enterica* et *E. coli*. En conclusion, cette étude a démontré que les biofilms obtenus ont des applications potentielles dans l'emballage alimentaire, en raison de leur capacité à préserver l'intégrité des aliments et à inhiber la croissance bactérienne.

Mots-clés :*Retama raetam*, galactomannane, biofilms, emballage de fromage, activité antibactérienne, activité antioxydant

Introduction

Introduction

The extensive use of plastic packaging in the food industry poses significant environmental challenges due to its non-renewable nature and lack of biodegradability. These plastics significantly contribute to solid waste generation, with plastic products accounting for approximately 12% of the global solid waste (2.01 billion metric tons in 2016) **(Ribeiro et *al*., 2021).** The dominance of plastic materials in the market is attributed to several factors, including low production costs, ease of transport, and lightweight properties **(Porta et *al*., 2011; Ribeiro et *al*., 2021).**Recent research highlights the potential health risks posed by the interaction between food and packaging made from petrochemical-based materials. These plastics can contain heavy metals such as cadmium and mercury, which have carcinogenic effects **(Hammam, 2019).** Additionally, the incineration of plastic waste emits harmful gases such as sulfur oxides, nitrogen oxides, and carbon oxides, exacerbating air pollution **(Hammam, 2019).** With increasing environmental awareness, consumer demand for eco-friendly and health-conscious products is rising. According to a United Nations report, half of all fruits and vegetables are lost annually**(Singh, D. P., & Packirisamy, G 2022).** Industries are striving to reduce post-harvest losses using edible coatings as a promising alternative to traditional plastic packaging. These coatings, derived from renewable sources such as polysaccharidees, proteins, or lipids, are fully edible and biodegradable **(Kandasamy et *al*., 2021; Ribeiro et *al*., 2021).** In addition to extending the shelf life of food products, edible coatings offer several additional advantages. Their semi-permeable nature allows controlled gas exchange between the food and the environment, ensuring optimal preservation conditions **(Kandasamy et *al*., 2021).** Furthermore, these coatings can be integrated with various functional additives, including probiotics, antimicrobials, and antioxidants, enhancing food safety and quality **(Chaudhary et *al*., 2020).** Researchers are also exploring the development of aesthetically pleasing edible coatings by incorporating flavors, colorants, and sweeteners **(Kocira et *al*., 2021).**

The growing interest in identifying new natural sources for producing edible coatings has led to extensive research on the potential applications of these materials. Polysaccharidees such as starch, chitosan, carrageenan, and galactomannan have been among the most studied for edible film production. In recent years, particular attention has been given to galactomannan derived from leguminous seeds due to its intriguing properties compared to

other polysaccharidee sources **(Aydinli and Tutas, 2000; Cerqueira et *al*., 2009a; Mikkonen et *al*., 2007; Saurabh et *al*., 2013).** The physical and chemical properties of galactomannan vary among species, with the mannose/galactose ratio in the galactomannan chain being one of the key variables used to predict its final properties. These polysaccharidees are water-soluble, forming highly viscous and stable aqueous solutions **(Mikkonen et *al*., 2007).** Due to their diverse physical and chemical properties, galactomannan is a versatile material used in various applications, including stabilizers and emulsifiers **(Stephen et *al*., 2006).** They are non-toxic, allowing their use in the textile, pharmaceutical, biomedical, cosmetic, and food industries **(Kapoor and Srivastava, 2005; Saha and Bhattacharya, 2010).** The primary plant sources of galactomannan are carob gum (Ceratonia siliqua L.), guar (Cyamopsis tetragonolobus), tara (Caesalpinia spinosa), fenugreek (Trigonella foenum-graecum)**(Prajapati et *al*., 2013)**, and *Retama raetam* **(O. Ishurd et *al*., 2004)**Recent research has focused on characterizing galactomannan films in terms of their barrier, mechanical, and thermal properties, as well as the effects of plasticizers and oils on their properties and their potential applications in food products **(Aydinli and Tutas, 2000; Bozdemir and Tutas, 2003; Cerqueira et *al*., 2009a; Cerqueira et *al*., 2011).**

The main objectif of the current study is to effect an extraction, and physico-chemical characterization of the water-soluble polysaccharidees extracted from Retama raetam seeds and it application in cheeze packaging using edible biofilms. Therefore, we divided the work into two parts: the theoretical part consisting of three chapters, the first chapter deals with the study of the plant of Retama raetam and introduced the legume family, while the second chapter deals with the seeds polysaccharidees with a focus on galactomannan, and it applications and the third chapter deals with the edible biofilm, its components, types, and methods of application. The practical part includes the fourth chapter where we clarified the methods and materials used in the research, the last chapter presented the results and discussed them, and we concluded the study with a general conclusion and further insight to this reaserch.

Bibliographic part

CHAPTER I

Retama raetam

I. Retama raetam

I.1.The Legume Family

The legumes, scientifically known as Fabaceae, as defined by (**Quezel and Santa.,1963)** and (**Ozenda .,1983),** belong to the angiosperm group, constituting the largest family of dicotyledonous seed plants. Originating from the Rosaceae family, they encompass over 730 genera and 19 thousand species **(Morale., 2011)**. Plants in this family are characterized by mostly compound, alternate, pinnate leaves, often endowed with stipules. They bear typically simple or compound flowers, usually zygomorphic and papilionaceous, composed of five sepals and five petals. **Both Mahnane (2009) and Sylivie (2011)** emphasizes the significance of legumes as a crucial plant family. These plants exhibit a remarkable ability to fix atmospheric nitrogen through symbiotic bacteria of the genus *Rhizobium*, residing within their roots. Additionally, legume seeds contain a substantial number of proteins, serving as an economical alternative to meat. Legumes are classified among the most nutritionally rich plant families, making them valuable dietary resources for both animals and humans .

I.2.The genus Retama

Retama, is a genus of flowering shrubs belonging to the Fabaceae family. It is distributed across various regions, including North Africa, the Mediterranean basin, and the Arabian Peninsula (**Bouredja et *al*.,2011)**. The genus *Retama* comprises species represented by shrubs or small trees ranging from one to four meters in height. The branches exhibit xeromorphic characteristics, often devoid of leaves, as an adaptation to arid desert environments with limited water availability. The stems are robust, featuring thick outer walls covered with a resilient skin. The flowers are clustered, presenting either white or yellow hues depending on the species **(Sid Ahmed,H., 2021)**. In algeria, this genus comprises three species: *Retama raetam*, *Retama spherocarpa*, and *Retama monosperma* **(Mahnane ,w., 2010).** For the purpose of our research, we have chosen to focus on *Retama raetam*.

I.3. *Retama raetam (Forssk) Webb*

The *Retama raetam (Forssk) Webb* is a Saharan shrub measuring 1 to 3.5m in height, characterized by velvety branches, white flowers of 8-10 mm, with a standard equalling or exceeding the crest, and an undeveloped pod on its ventral side housing a small seed **(Sid Ahmed,H., 2021)**.

Figure 01: Branches of *Retama raetam* **(Kaoudja, K.,2022).**

Figure 02: *Retama raetam* flawers **(Malre., 1987).**

Figure 03: *Retama raetam (Forssk) Webb* **(Kaoudja, k., 2022).**

I.3.1.Geographical spread of the *Retama raetam (Forssk) Webb*

I.3.1.1. Geographical spread of the *Retama* plant in the world

The geographical distribution of *Retama raetam (Forssk) Webb* worldwide is notable, with its presence predominantly in the Sahara Desert of North Africa. The plant has been observed in maritime sands along the Mediterranean coast, thriving in dunes and sandy slopes in countries such as Algeria, Tunisia, Morocco, Libya, and Egypt. Additionally, it extends its

distribution to temperate Asian countries like Palestine, Lebanon, and Jordan, as well as in parts of Eastern and Southern Europe **(Hawka and Margheni., 2016).**

I.3.1.2 Geographical spread of the *Retama* plant in the Algeria

The geographical distribution of the *Retama raetam (Forssk.) Webb* plant in Algeria encompasses sandy terrains, notably observed in the regions of El Oued, Ouargla, Tebessa, Biskra, Ghardaïa, Laghouat, Ain Sefra, El Bayadh, and the Kabylie Mountains in southern Oran. The *Retama raetam (Forssk) Webb* species thrives specifically in arid environments, contributing to its prevalence in these sandy lands across various algerian provinces **(Hawka and Margheni., 2016)**.

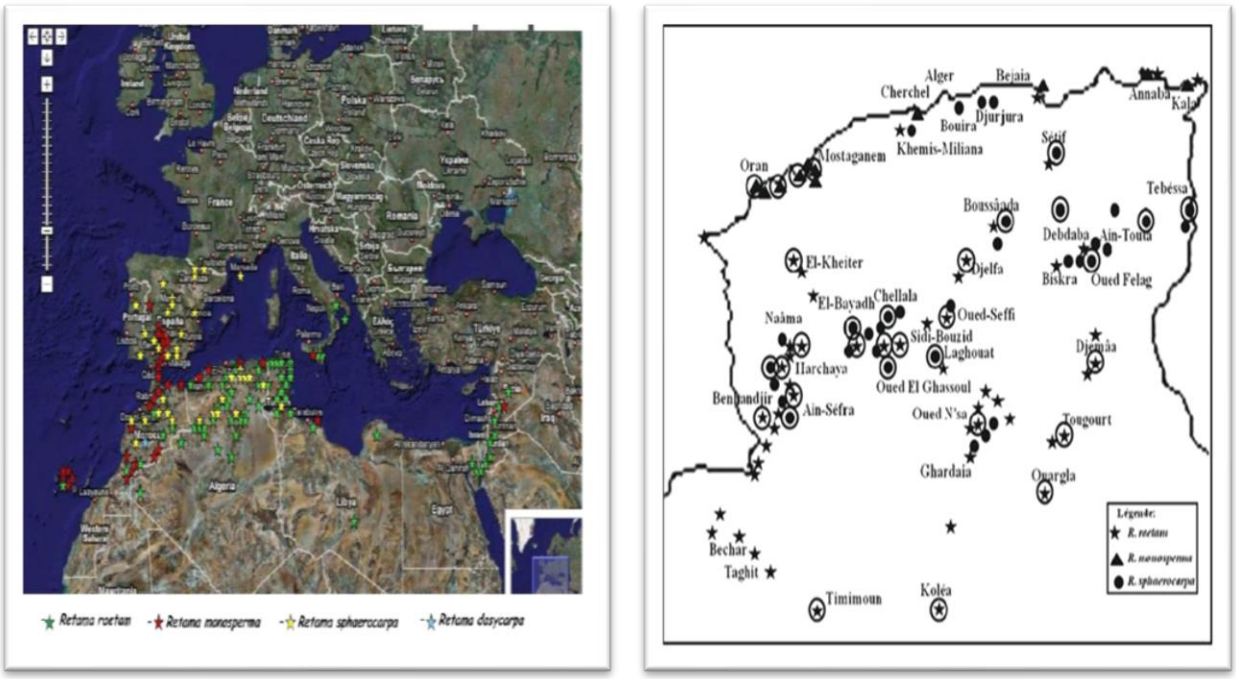


Figure 04: Geographical spread of the *Retama raetam (Forssk) Webb* in the Algeria and in the world *(***Benmiloud-Mahieddine., 2013).**

I.3.2. Importances and applications of *Retama Raetam (Forssk) Webb*

Due to its wide geographic distribution spanning from the Mediterranean coast to semi-arid and arid regions, *Retama raetam (Forssk) Webb* exhibits both pharmacological and ecological significance.

I.3.2.1. Ecological interest

The ecological significance of *Retama* plays a pivotal role in maintaining the equilibrium of natural environments and ecosystems, particularly in arid and semi-arid zones. Acknowledged as plants well-suited to harsh conditions, *Retama* species exhibit adaptability

to extreme drought and salinity through their xeromorphic botanical morphology and structure **(Mostefai and Mansouri., 2020)**. According to (**Mittler et *al*., 2001)**, *Retama Raetam (Forssk) webb* demonstrates resilience to the most challenging climatic changes, such as nutrient deficiency and water stress. **(Ferchichi., 1996)** suggests that, due to its high germinative potential, tolerance to water stress, and radial branching pattern, *Retama Raetam* qualifies as a pioneering species capable of colonizing dune systems. Consequently, its utilization in vegetation operations within these fragile environments is highly recommended.

I.3.2.2.Economic significance

The North African population extensively utilizes this species for construction and ornamentation, as well as for the treatment or improvement of various ailments. *Retama* plant species are also employed in pastures to provide shade and shelter for animals, especially during hot and dry days **(Leon-Gonzalez et *al*., 2018**).

I.3.2.3.Agro-climatic Importance

Retama thrives in a diverse range of habitats, showcasing resilience to low winter temperatures and extremely hot summer condition **(Barakat ,N et *al*., 2013).**

I.3.3. Use in traditional medicine

Retama raetam finds application in traditional medicine, commonly referred to as "*R'tam."* Its use extends to mitigating skin inflammation in popular medicine, addressing joint pains in Lebanon's herbal tradition, and combating skin diseases in the Grand Maghreb region. Prior pharmacological studies on this plant have unveiled its multifaceted medicinal properties, including antibacterial, antifungal, anti-hypertensive, antioxidant, antiviral, diuretic, hepatoprotective, nephroprotective, and cytotoxic effects **(Dziri et *al*., 2012; Djeddi et *al*., 2013)**. A decoction of *Retama retam* roots or leaves is employed for diabetes management. The infusion of fresh *Retama raetam* leaves serves as a footbath, inducing hypoglycemia **(Benkhnigue et *al*., 2014)**. However, caution is advised with the potentially toxic *Retam* fruits, known for inducing hallucinations **(Trabut., 2006)**.

I.4. Intraspecific taxon:

In accordance with the species profile on the IUCN Red List **(Roskov et *al*., 2020)**, two primary intraspecific taxa are identified: *Retama raetam subsp, Rataem* and *Retama raetam subsp,Gussonei (Webb) Greuter*.

Conversely, the Kew Botanical Garden **(POWO., 2020)** delineates three intraspecific taxa: *Retama raetam subsp. Bovei (Spach) Talavera & P.E. Gibbs, Retama raetam subsp. Rataem,*

and *Retama raetam subsp. Gussonei (Webb) Greuter.* The taxonomic classification is as follows**:**

Table 01: Classification of *Retama raetam (Forssk) Webb* ***(AL-Sharari* et *al., 2020).***

Kingdom:	*Plantae – Plants*
Subkingdom:	*Vascular plants*
Superdivision:	*Seed Plants*
Division	*Flowering plants*
Class	*Dicotyledons*
Subclass	*Rosidae*
Order	*Fabales*
Familly	*Fabaceae / Leguminosae*
Genus	*Retama Raf*
Species	*Retama raetam (Forssk.) Webb & Berthel*

I.5. Chemical compositions of *Retama raetam*

I.5.1. Alkaloids

Through a study to evaluate the alkaloids of *Retama Raetam (Forssk) Webb*, the results of chemical detection showed that the *Retama Raetam (Forssk) Webb* plant contains alkaloids in its various parts (flowers, fruits, roots) in both the Dragendoff test, the Bouchard test, and also the Mayer test. It has proven its effectiveness in relieving pain, and the same results were also obtained in other previous studies on the same plant, such as the study conducted by (**Ben Sassi.H., 2018)**, a study of the biological effectiveness of different extracts of the Stom and Drain plants. *Retama* is characterized by a quinolizidine alkaloid **(Zekri.R and Sasui.R.,2019).**

The fruits of *Retama raetam (Forssk) Webb* are considered highly toxic due to their alkaloid content, which induces hallucinations upon ingestion. Additionally, in some cases, consumption may lead to miscarriage or poisoning **(Benhouhou., 2005).**

1.5.2. Flavinoids

As a result of studying the flavonoids of the ethyl acetate extract of the *Retama raetam (Forssk) webb* plant using high-performance chromatographic separation methods, it was proven that the plant contains all the active compounds, the most important of which are flavinoids and glycoflavinoids. Since flavinoids are phenolic substances, they have an activity against oxidation that lies in their capture of free radicals, meaning that they bind to them and thus to their structural formula **(Kaoudja, k., 2022) .**

I.5.3. Tannins

In a study conducted in which tannin was detected and the test was positive, this indicates the presence of tannin in the *Retama* plant. *Retam* is usually considered a compound that contains polyphenols and is known for its antioxidant, antimicrobial and anti-inflammatory properties **(Ben sassi.H., 2018).**

I.5.4. Saponins

In a study of the biological effectiveness of different extracts of the saprophyte and saprophytic plants, saponins were detected, and through the positive results that appeared, it was confirmed that the saprum plant contains saponins **(Ben sassi. H., 2018).**

I.5.5 Essential oils

In a previous study, essentiel oils were detected, and a change in color was observed from the results, which indicates the presence of oils in the *Retama raetam (Forssk) Webb* **(Ben sassi.H., 2018).**

I.5.6. Polysaccharide

The polysaccharides found in plants comprise a diverse group of chemical compounds that play vital roles in plant growth, development, and nutrition. These sugars include glucose, fructose, sucrose, and other oligosaccharides, generated through the process of photosynthesis occurring in the leaves utilizing light, carbon dioxide, and water. Sugars are stored in various parts of the plant, such as roots, fruits, and seeds, serving as a source of energy, building blocks, and storage. Among these polysaccharides is galactomannan, where galactomannans found in seeds represent a diverse class of sugars ubiquitous in nature.

Galactomannan was extracted from *Retama raetam (Forssk) Webb* seeds according to the **(Cerqueira et *al*., 2009)**.

In another study, the physical, chemical, surface, and emulsification properties of *Retama raetam* were investigated. *Guar* galactomannans were relatively well studied. The results indicated that *Retama raetam* galactomannan consists primarily of total carbohydrates (95.52%) with a lower protein content (0.87%). The sugars identified were mannose (Man) and galactose (Gal), with a Man: Gal ratio of (1.85%) compared to *Guar gum* (1.83%). Thermal property results showed similar glass transition temperature (Tg) and melting temperature ™ to those found in *Guar gum*. **(Chouaibi, M et *al*., 2018).**

Table 02: Previous studies of *Retama raetam* plant.

Used part	Extraction method	Activity	Reference
Papers	The plant leaves are steeped in boiling water, the resulting solution is filtered, and then used as compresses applied daily to the affected area.	Stop bleeding and treat wounds	**(Zekri.R and Sasui.R ., 2019).**
Flowers	The essential oil was extracted by hydrodistillation of the dried plant material (100 g of each sample in 500 mL of distilled water) using a Clevenger-type apparatus for 4 h.	Antibacterial, antifungal and antioxidant activities of the flower oil of *Retama raetam.*	**(Edziri.H et *al.*, 2010).**
Fruits and seeds	Extracted with methanol at room temperature using IKA ULTRA-TURRAX T 25 digital (Janke and Kunkel, IKA Labortechnik, Stauten, Germany) according to the manufacturer's instructions.	The antihyperglycemic activity of *Retama raetam* in streptozotocin-induced diabetic rats	**(Mardi M. Algandaby., 2010).**
Legs	Grind the dried stems into a powder and place it on the affected area	It is used to stop bleeding	**(Zekri.R and Sasui R., 2019)**
The roots	Three grams of the roots are boiled in 100 milliliters of distilled water for 10 minutes. After filtering out the microorganisms, the extracts are evaporated to dryness under vacuum using a rotary evaporator.	Analgesic	**(Djeddi et *al.*, 2013)**

CHAPTER II

Seeds storage Polysaccharides

II. Seeds storage Polysaccharides

II.1. Overview

Cell wall storage Polysaccharides(CWSPs) are important storage compounds in the seeds of many important plant groups, which have evolved efficient mechanisms to break down these cell walls for growth. These seeds exhibit high non-cellulosic polysaccharid activities. Research on CWSPs can provide insights into polysaccharid deposition during seed maturation and control the source-sink relationship in seedling development, providing potential biotechnological applications **(Buckeridge. M. S.,2010)** Due to their compelling physicochemical and functional properties **(Ganter and Reicher, 1999)** water-soluble Polysaccharides exhibit remarkable thickening, crystallization, stabilization and emulsification properties, classified within the category of dietary fiber, thus not only enhancing sensory (rheological) properties but also facilitating the production of foods with nutritional properties. The incorporation of these polymers into various foodstuffs has gained significant traction, driven by increasing consumer demands for health-promoting foods (low in calories, rich in dietary fiber and bioactive molecules. These hydrocolloids exhibit a high degree of hydrophilicity and flexibility, giving them exceptional water-binding capabilities. It can be extracted from plant sources (such as *gum arabic*, *guar*, *carob*, and pectin). Among the Polysaccharides stored in plant seeds are mannan, glucomannan, or galactomannan, and galactomannans represent a large subset of hydrocolloidal Polysaccharides**,** extracted primarily from the endosperm of legumes. Especially *carob* and *guar gum*. Characterized by their abundance, non-toxicity, and biodegradability, these polymers have been recognized for their binding properties since ancient times, For example, the Egyptians used carob gum to adhere mummy bandages. However, galactomannans did not appear as industrial products until the 20th century. Current research on the characterization of the structure, modifications and applications of galactomannan is prolific, as evidenced by the continuous expansion of the scientific literature related to these biopolymers **(Sadoud., 2013).**

II.2. Definition

Galactomannans (GM) are hydrophilic and neutral Polysaccharides, extracted from the endosperm of approximately 70 legume species and the cell wall of certain bacteria **(Dakia et *al*., 2009).** Serving as plant reserve Polysaccharides **(Grisel et *al*., 2015)**, they consist of a main chain of D-mannopyranose residues linked in β-(1 4) with unique D-galactopyranose residues branching off via an α-(1 6) linkage (Figure 05). Variations among them lie in the mannose/galactose (M/G) ratio. The degree of galactomannan branching, expressed by the

M/G ratio, depends on the plant, its source, and the extraction method employed. This part belong to the composition not definition even the figure 05 **(Guemmane and Kerfal., 2016).**

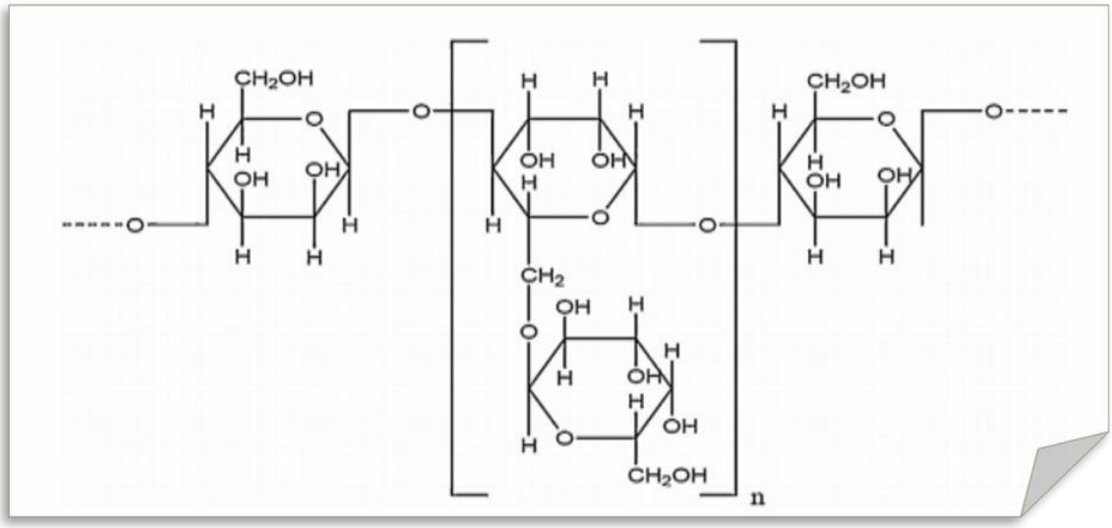


Figure 05: General molecular structure of a galactomannan **(Prajapati et *al*., 2013).**

II.3. Chemical composition

Galactomannan is a polymer consisting of an α-D-mannopyranosyl backbone linked (1→4), substituted at O-6 with single β-D-galactopyranose units. The mannose to galactose ratio (M/G) varies from approximately 10 to 1, depending on the plant source. The substitution of α-D-galactopyranose residues in the side chains has a crucial impact on solubility. Solubility in water increases with higher galactose content (i.e., lower M/G ratio), likely because galactose substitutions inhibit the solid-state packing of mannan chains and contribute to conformational entropy in solution by allowing free rotation around the (1→6) linkages. A 10% galactose substitution level is essential for water solubility. Galactomannan with less than 10% galactose precipitates at room temperature due to the association of unsubstituted regions of the polymer figure 06. At lower temperatures, solubility is restricted to chains with a higher galactose content than the average for the entire gum, and fractions with different M/G ratios can be obtained by dissolving at progressively higher temperatures(**Silveira et *al*., 2011).**

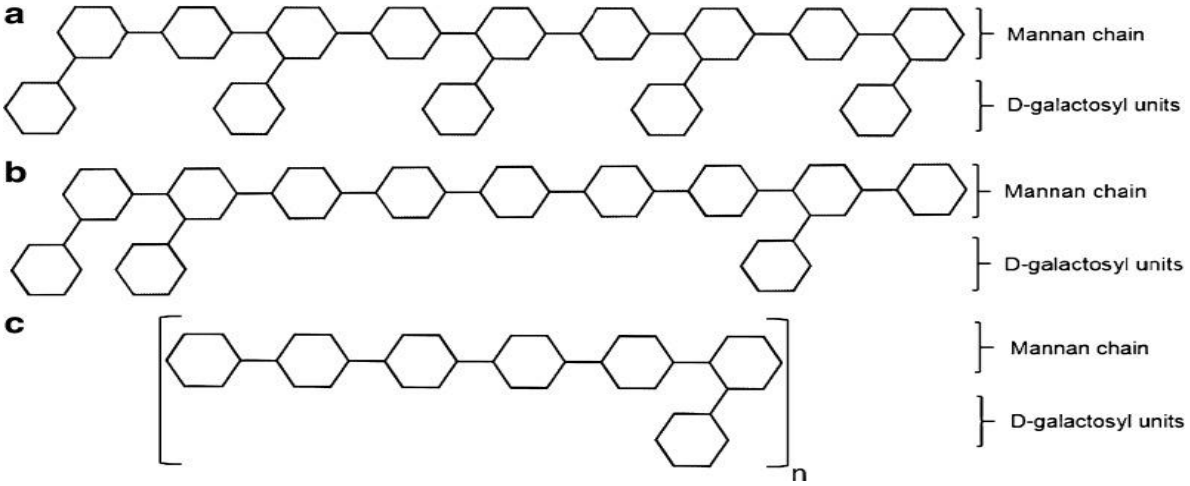


Figure 06: Schematic representation of typical distribution of D-galactose units along the mannan chain in three **(V.R.F.Dos sant et *al.*, 2015).**

II.4. Main sources

II.4.1. *Fenugreek gum*

Fenugreek (Trigonella foenum-graecum), a legume indigenous to the Mediterranean area, harbors galactomannan (GM) with a G/M ratio nearing 1:1.41. This polysaccharid is water-soluble at temperatures below 20°C and yields solutions exhibiting reduced viscoscompared to counterparts at similar concentrations. Additionally, this galactomannan exhibits emulsification capabilities (**Chouana et *al.*, 2017)**.

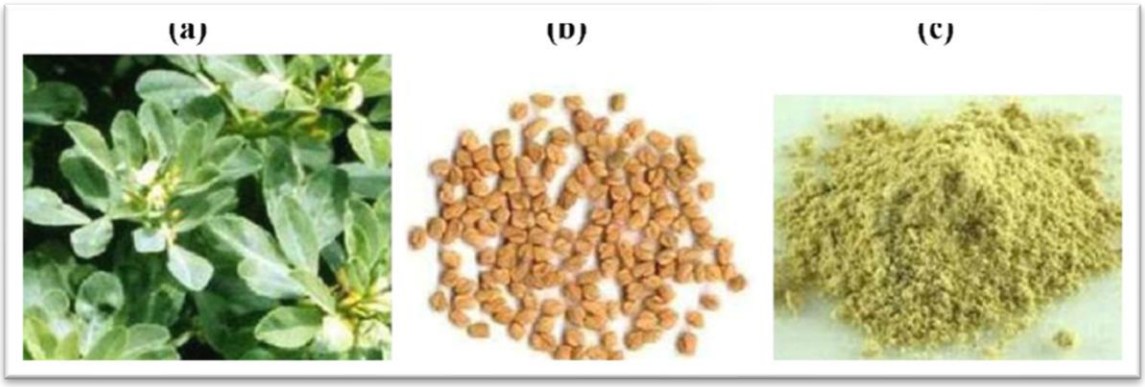


Figure 07: *Fenugreek gum* (a) plant, (b) seeds and (c) flour **(Prajapati et *al.*, 2013).**

II.4.2. *Guar gum*

Guar gum (depicted in Figure 08) originates predominantly from the southern regions of the United States. Its historical application as a food additive traces back to India. Post-World War II, *guar gum* emerged commercially, emerging as a viable alternative to *locust bean gum*. Structurally, *guar* presents as a linear mannan, characterized by a higher incidence of branched galactoses compared to *locust bean gum* (with a G/M ratio of 1:*Tara gum* (depicted in Figure 09) exhibits a G/M ratio of 1:3, falling between the ratios observed in *locust bean gum* and *guar gum*. The rheological characteristics of *tara gum* solutions closely resemble those of *locust bean gum*. Despite its availability in the market, lacks discernible niche applications and primarily serves as a substitute for other galactomannans (GMs).2), facilitating enhanced solubility in water, even below 20 °C. Rheologically, *guar gum* mirrors *locust bean gum*, showcasing pseudoplastic behavior that diminishes with rising temperatures, while maintaining stability across a broad pH spectrum. Similar to *locust bean gum*, its pairing with certain Polysaccharides like xanthan can induce gel formation, albeit with lesser rigidity due to diminished non-branched regions, paralleling conditions observed with *locust bean gum*. The utility of *guar gum* mirrors that of *locust bean gum* (**Chouana., 2017)**.

Figure 08: *Guar gum* (a) pods, (b) seeds and (c) flour **(Prajapati et *al*., 2013).**

II.4.3. *Tara gum*

Tara gum (depicted in Figure 09) exhibits a G/M ratio of 1:3, falling between the ratios observed in *locust bean gum* and *guar gum*. The rheological characteristics of *tara gum* solutions closely resemble those of *locust bean gum*. Despite its availability in the market, *tara gum* lacks discernible niche applications and primarily serves as a substitute for other galactomannans (GMs) (**Chouana., 2017)**.

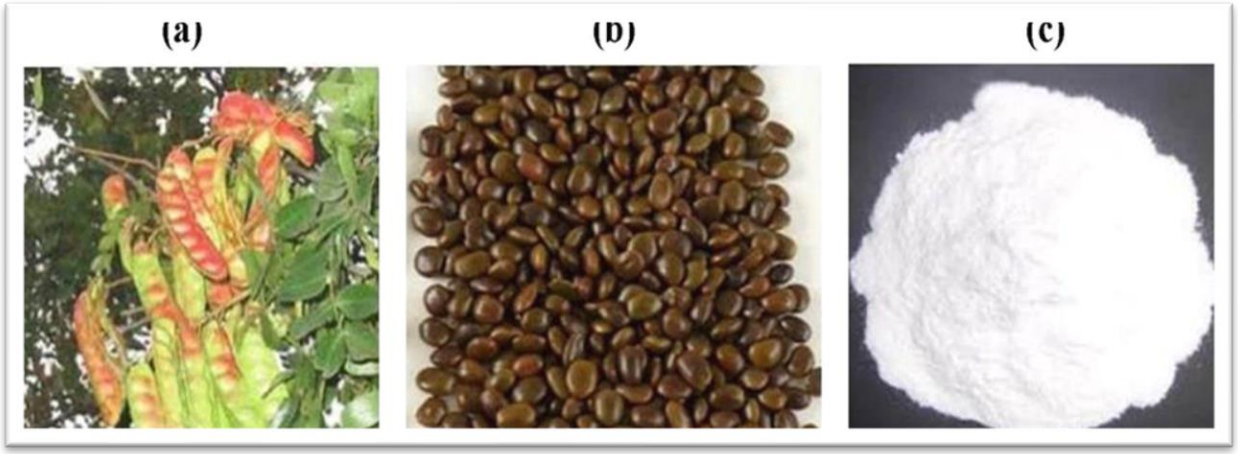


Figure 09: *Tara gum*. (a) pods, (b) seeds and (c) flour **(Prajapati et *al*., 2013).**

II.4.4. *Locust bean gum*

In the Mediterranean and the Middle East, *locust bean gum* finds application in traditional culinary practices. It is worth noting that its therapeutic properties trace back to antiquity. The pods of this plant (Figure10) are combined with various ingredients including honey, oatmeal, and wax to address ailments such as diarrhea, eye infections, visual impairments, and intestinal parasite infestations. In Morocco, Berbers consume *carob* tree fruits in beverage form to alleviate digestive issues. Additionally, in certain regions, *carob* flour serves as a substitute for wheat flour in powdered milk for infants due to its low allergenicity and high nutritional value **(Silveira et *al*., 2011).** *Locust bean gum* boasts a (G/M) ratio of 1:4 and a molecular mass of approximately 30 x 10^4 g/mol. The rheological behavior of this gum exhibits typical pseudoplastic characteristics, diminishing with decreasing temperature **(Gillet., 2014).** Its solubility in cold water is minimal. Elevated temperatures facilitate the solubilization of polymers abundant in "smooth zones," characterized by limited branching, promoting aggregation and entanglement, thus forming dense networks and highly viscous solutions, even at low concentrations, due to restricted interactions with water molecules **(Gillet., 2014).** Moreover, it serves as a widely utilized thickening and stabilizing agent in numerous food products, including ice creams, spreads, salad dressings, and charcuterie items.

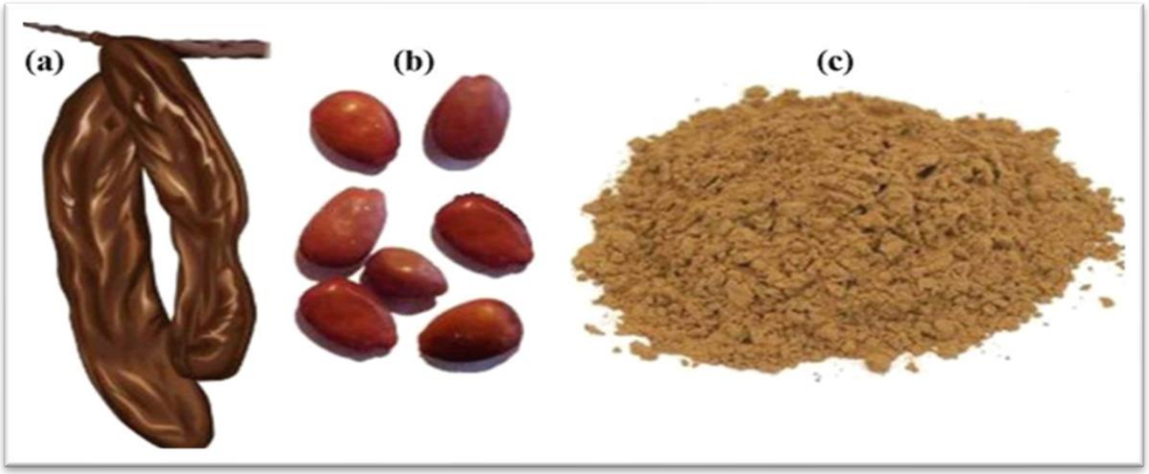


Figure10: *Locust bean gum.* (a) pods, (b) seeds and (c) flour **(Prajapati et *al.*, 2013).**

II.4.5. *Cassia gum*

Cassia gum is primarily sourced from the endosperm of Cassia obtusifolia and Cassia tora, members of the Leguminosae family indigenous to subtropical regions globally. Additional species yielding *cassia* galactomannan include *Cassia angustifolia, Cassia javahikai, Cassia pleurocarpa, and Cassia javanica* **(Mirhosseini and Amid., 2012). (Chaubey and Kapoor., 2001)** analyzed the compositional parameters of *Cassia angustifolia*, revealing that the cassia seed comprises the seed coat (20–24%), endosperm (48–52%), and germ (28–32%). The gum derived from this seed contains 10.6% protein, 4.1% ash, 5.8% ether extract, and 34% water-soluble galactomannans, among other constituents. The M/G ratio and molecular weight of galactomannans from *Cassia* seed very among species. For instance, in *Cassia tora* and *Cassia obtusifolia*, the M/G ratio was 5:1, with molecular weights ranging from 2 105 to 3 105 Da **(Mahungu and Meyland., 2008).** Conversely, in *Cassia angustifolia*, the M/G ratio was 2.9, with an average molecular weight of 9.66 × 104 Da **(Chaubey and Kapoor., 2001).** Polymers derived from *Cassia gum* extracted from *Cassia javahikai* exhibit biocompatibility, biodegradability, and are widely utilized as hydrocolloids **(Singh, Srivastava, and Tiwari., 2009b)**. The nonionic galactomannan isolated from *Cassia pleurocarpa* demonstrates notable water retention capacity, viscosity-enhancing properties, and unique gelling abilities, rendering them excellent substrates for tissue engineering, biomedical applications, and drug delivery.

Figure 11: Different parts of *Cassia tora* plant (A) Shoot part of the plant (B) Flower (C) Pod with the seed of *Cassia tora* plant **(Palmei et *al*., 2021).**

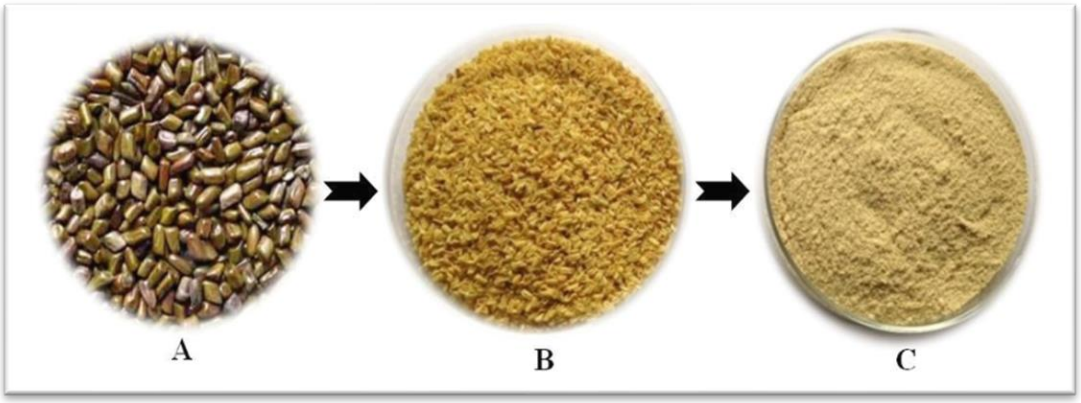


Figure 12: *Cassia gum* powder isolated from the seed of the plant. (A) Seed of *Cassia tora* (B) The endosperm (split) (C) *Cassia gum* powder **(Palmei et *al*., 2021).**

II.5. Physico-chemical properties

II.5.1. Influence on solution and viscosity

Galactomannans are commercially distributed and stored in powdered form. Upon dissolution, macromolecules with higher galactose content exhibit increased solubility at lower temperatures. For instance, *guar* (M/G = 2:1) with 40% galactose achieves maximum solubility and viscosity at 40°C. Conversely, macromolecules with minimal branching resist dissolution, potentially forming polymer clusters that are challenging for solvents to access. Consequently, heating, accompanied by vigorous stirring, becomes necessary for maximal solubilization and viscosity, as observed with *Carob* (M/G = 4:1) containing 20% galactose, reaching peak solubility and viscosity at 80°C. These findings align with various studies **(Dakia et *al*., 2010).**

II.5.2. Solubility property

The solubility of galactomannans in water is contingent upon their galactose content, with increasing levels of this sugar correlating with heightened solubility. Galactopyranose residues within the galactomannan molecule confer hydrophilic characteristics, thereby augmenting water solubility as the degree of galactose substitution rises. These galactose side chains facilitate the extension of mannan chains and prevent the formation of insoluble associations; in the absence of galactopyranose groups, pure mannans exhibit complete insolubility in water. The absence of these galactopyranose moieties renders the polysaccharid susceptible to crystallization or precipitation in solution**.** Macromolecules rich in galactose residues demonstrate enhanced solubility at lower temperatures; for instance, *guar gum (*with a M/G ratio of 1.6) achieves maximal solubility at 40°C. Conversely, macromolecules with limited or absent branching exhibit resistance to dissolution, potentially forming polymer clusters. Consequently, a heat treatment involving vigorous stirring (e.g., heating at 85°C for 10 minutes) is essential for complete solubilization, as seen with *locust bean gum* characterized by a lower substitution level (M/G ratio of 3.5 to 4). Galactomannans remain insoluble in organic solvents but can be precipitated from aqueous solutions through the addition of water-miscible solvents like ethanol **(Sadoud., 2013).**

II.5.3. Viscosity property

Viscosity stands as the paramount quality parameter for thickening agents. Leveraging their water-binding prowess, galactomannans demonstrate the ability to yield highly viscous solutions at minimal concentrations (0.5-1%). The intrinsic viscosity (η), denoted in ml.g-1, delineates the specific hydrodynamic volume occupied by a unit mass of polymer in a given solvent, ascertained at 25 °C within a 1% galactomannan solution utilizing a viscometer. This metric's value is influenced by both intramolecular (molecule self-folding) and intermolecular (molecule-molecule association) interactions within the unsubstituted zones of galactomannans, facilitated by hydrogen bonding. Consequently, galactomannans boasting a high mannose to galactose (M/G) ratio, indicative of low galactose residue content, exhibit heightened viscosity. The M/G ratio significantly impacts galactomannan solubility in water and the viscosity-concentration relationship within the solution. The aggregation phenomenon, pivotal in governing the properties of these water-soluble polymers, originates from cooperative hydrogen bonding between segments of chains lacking galactose residues. As a result of these bonds, galactose-deficient galactomannans exhibit superior viscosity compared to heavily substituted variants. However, they encounter challenges in solubilization unlike galactomannans rich in galactose **(Sadoud., 2013).**

II.5.4. Stability property

The depolymerization of the mannan chain results in a notable decrease in viscosity, as observed in scenarios such as the heating of galactomannan solutions at very low pH levels. These solutions exhibit stability within a pH range of 3 to 11 at room temperature but are susceptible to degradation by microorganisms, necessitating sterilization before storage**.** Solutions containing guar and tara gums demonstrate excellent stability during freezing-thawing cycles, whereas *carob gum* partially becomes insoluble after freezing **(Sadoud., 2013).**

II.5.5. Galactomannan mobilization and control of M/G ratio

During seed maturation, galactomannans undergo accumulation within the endosperm cellular matrix , progressively displacing the protoplast. This deposition process persists until the protoplast undergoes degeneration. Subsequently, the inert galactomannan matrix becomes enveloped by a layer of viable cells termed the aleurone layer. Upon commencement of seed germination, hydrolytic enzymes synthesized by aleurone cells catalyze the breakdown of galactomannan. These enzymes encompass α-galactosidase, endo β-mannanase, and β-mannosidase, which collectively facilitate the cleavage of galactose side chains and the fragmentation of the mannose backbone into oligosaccharids and mannose units. A-Galactosidases are prominently present in leguminous seeds, being augmented by aleurone cells during germination. Investigations into enzyme kinetics and substrate specificity have unveiled variances across diverse legume species, thereby influencing galactomannan metabolism dynamics. The functional role of α-galactosidase in modulating the galactosylation extent along the mannan backbone has been further elucidated through genetic expression analyses. Notably, in coconut, the absence of α-galactosidase precipitates the formation of galactomannan with a diminished M/G ratio compared to wild-types. The elucidation of the α-galactosidase gene sequence from *guar* plants has illuminated its regulatory role in post-depositional processes, thereby shaping the galactosylation pattern. Furthermore, β-mannosidases participate in the breakdown of galactomannan. The enzymatic hydrolysis of galactomannan yields monosaccharid constituents, predominantly galactose and mannose, which serve as substrates for sucrose biosynthesis in the endosperm. Interrelations between starch biosynthesis in cotyledons and the degradation of cell wall Polysaccharides may be intertwined. The metabolic fate of galactomannan degradation products implicates the involvement of phosphomannoisomerase and phosphoglucoisomerase enzymes in catalyzing the isomerization of mannose to glucose, thereby contributing to sucrose synthesis in *guar* seed endosperm **(Ramakrishna.G. et *al.*, 2020).**

II.5.6.Interaction with other Polysaccharides

The interplay with other Polysaccharides stands as a captivating characteristic of galactomannans**.** Combining this polysaccharid with κ-carrageenan, *xanthan gum*, or agar elicits a concentration-dependent synergistic effect, yielding highly viscous solutions**.** Upon heating, solutions of LBG and *xanthan gum* develop robust elastic gels upon cooling. This synergy arises from the establishment of a three-dimensional polysaccharid matrix facilitated by the formation of junction zones between *xanthan gum* and the unsubstituted regions of LBG. Galactomannans featuring a high galactose content exhibit diminished synergistic effects on gelation**.** Comparable phenomena manifest with κ-carrageenan, where solo application yields less viscous gels, but with the addition of LBG, elastic and cohesive gels form. It's postulated that galactomannan chains with frequent longer soft regions promote favored synergistic interactions**.** Moreover, the galactose content governs the mode of interaction with other polymers such as pectins, soy proteins, starch, agarose, cellulose, gelatins, carrageenans (κ and λ), β, κ, and α-caseins, agar, and xanthan**.** The strength of these interactions amplifies with decreasing galactose content in galactomannan. Consequently, *locust bean gum* displays superior interaction compared to *guar gum* with carrageenans, yielding more consistent and elastic gels**.** This partnership with carrageenans is explored for gelatin substitutes. Furthermore, *locust bean gum* and *xanthan* exhibit synergistic behavior, forming thermoreversible gels that maintain stability upon freezing. The proposed model posits interactions between carrageenans and galactomannans, involving double helices of carrageenan and the "smooth" zones along the mannan chain, reinforcing the gel's cohesion**.** Notably, *xanthan gum*, akin to *carob*, lacks gelation capability independently but demonstrates formidable thickening properties. However, a blend of these gums, post-heating and cooling, results in an elastic gel, facilitated by a conformational transition of xanthan that allows it to associate with the "smooth" regions of galactomannans **(Sadoud., 2013).**

II.6. Application

Galactomannans they offer cost-effective, environmentally friendly, and non-toxic solutions. These polyannans, present in seeds, represent a diverse class of Polysaccharides distributed widely in nature Polysaccharides exhibit a range of chemical properties, rendering them versatile materials for various applications. Among the notable properties of seed galactomannans are water retention, thickening, emulsification, binding, suspension, crystallization, film formation, and applications In the pharmaceutical industry. Galactomannans find diverse applications across cosmetics, food, drilling, explosives, paper, petroleum, pharmaceuticals, and textiles**.** Their bonding properties can be enhanced through

interactions with monomers and polymers, leveraging the synergistic interactions of multiple hydroxyl groups (-OH). Additionally, their gelatinous nature allows for various forms suitable for human consumption **(Lavudi et *al*., 2020).**

II.6.1. Industrial Applications

A Galactomannans exhibit multifaceted utility across various sectors including rubber, thermoplastics, food, cosmetics, and toiletries. They serve as essential components in toothpaste formulations as thickeners, shampoos as conditioners, and textile dyes. Moreover, they find application in denture fixture powders and are integral in the sizing and finishing processes within the printing industry. Notably, galactomannans augment sheet formation, facilitate folding, and contribute to the development of dense printing surfaces **(Lavudi et *al*., 2020).**

II.6.2. Pharmaceutical Industries

Galactomannans serve as effective surface coating agents and binders, along with functioning as disintegrators for tablet preparation**.** They also play a pivotal role in drug formulations. Primarily utilized as a component in bulk-forming laxatives, they exhibit therapeutic potential in treating inflammatory conditions**.** It's readily engage with other compounds, thereby enhancing tablet preparation and serving as additives in the pharmaceutical industry. Over the past two decades, galactomannan-based nanoparticles have undergone significant advancements and thorough exploration for pharmaceutical purposes **(Lavudi et *al*., 2020).**

II.6.3.Food industries

Galactomannans exhibit versatile applications in the food industry, serving as essential components for various functions such as food coating, adhesive properties, and enhancing the texture of products like ice creams and processed foods.

They function effectively as thickeners in creams and in the production of milk-based desserts within dairy operations, as stabilizers in sorbets, ice creams, and cheese manufacturing. With their high moisture retention capabilities, they not only improve flavor but also serve as substitutes for fats. Furthermore, they play a significant role in the preparation of diabetic-friendly products such as coffee whiteners and baby milk formulations, as well as enhancing the quality of bakery products, particularly bread. Galactomannans find applications in a wide array of products including powdered mixes, desserts, hot milk puddings, jellied products, syrups, cured meats, frozen and canned meats,

and beverages, where they serve as thickeners and enhance the overall quality of dairy products **(Lavudi et *al*., 2020).**

CHAPTER III

Edible biofilm

III. Edible biofilm

III.1. Overview

Food packaging serves a crucial role in safeguarding produce from biological, physical, and chemical hazards throughout the supply chain **(Chettri et *al*., 2023).** A multitude of polysaccharides, including chitosan, carrageenan, starch, cellulose, and their derivatives, have been explored for the creation of biodegradable films. Notably, galactomannan, a type of plant polysaccharide, exhibits promising potential as a raw material for film formation. Furthermore, galactomannan possesses the advantageous properties of being suitable for human consumption **(Zhao et *al*., 2019).**

III.2. Definition

Edible films and coatings are characterized as thin materials utilized for enveloping or encasing products to prolong their shelf life, while also being consumable alongside the product**(Kocira et *al*., 2021).** The constituents of these films and coatings, including hydrocolloids or natural gums (comprising proteins, polysaccharides, or alginates), as well as lipids (consisting of fatty acids, acylglycerols, or waxes),are taken into account **(Singh, D. P., &Packirisamy, G., 2022).** Polysaccharide-based films and coatings exhibit colorlessness, impart an oil-free appearance, and possess a minimal caloric content. These materials hold promise for extending the shelf life of perishable items such as fruits, vegetables, shellfish, and meats by effectively mitigating dehydration, surface darkening, and oxidative rancidity.

Furthermore, specific polysaccharides employed in edible packaging function as carriers for organic acids, which act as inhibitors against common foodborne pathogens such as *Listeria monocytogenes, Salmonella,* and *Escherichia coli.* Additionally, polysaccharide edible films demonstrate antioxidative properties and can function as functional supplements, serving as nutraceuticals within the product. Beyond their environmentally friendly nature, many of these novel edible materials serve as rich sources of vitamins and minerals while also enhancing the overall sensory profile of food products **(Nešić et *al*., 2019).**The thin layer formed by the edible materials that envelop fruits and vegetables is termed the edible coating, whereas the pre-formed layer applied to the raw material is referred to as the edible film. These coatings exist in liquid form and are administered to the raw material through immersion in the solution **(Kocira et *al*., 2021).**

III.3. Composition

Biopolymers exhibit dual sustainability characteristics, being both biodegradable and sourced from renewable reservoirs. Originating from natural sources, these polymers undergo cycles of utilization and decomposition, exemplified in Figure 13. This cyclic process underscores their potential to perpetually renew and integrate seamlessly within ecological systems, transitioning from one phase to the next in an uninterrupted continuum **(Chen et *al*., 2019).** where Biopolymers, encompassing polysaccharides, proteins, and lipids, offer versatile applications in the creation of edible films and coatings **(Tavassoli-Kafrani et *al*., 2016).**

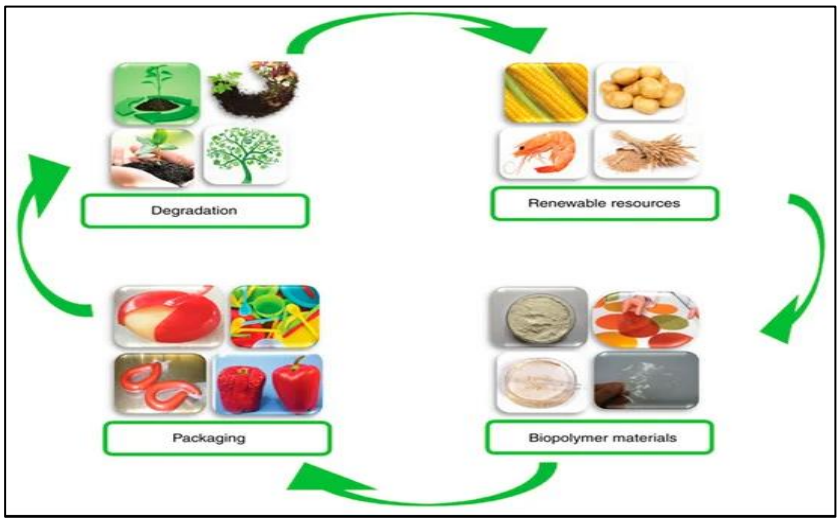


Figure 13: The life cycle pertaining to biopolymer packaging materials **(Chen et *al*., 2019).**

Traditionally, edible films and coatings have predominantly consisted of single-component formulations. However, recent research has delved into the realm of two-component and multi-component edible materials, offering enhanced functional attributes. Composite films or coatings are crafted by blending two or more binders to create structures with modified physical, mechanical, and barrier properties surpassing those of single-component materials. Consequently, a variety of substances including plasticizers (such as glycerol, polyoxyethylene glycol, propylene glycol, sorbitol), cross-linkers, emulsifiers, and enhancers (like vegetable fatty acids) are incorporated into film-forming formulations to enhance or alter the fundamental properties of the material **(Kocira et *al*., 2021).**

III.3.1.Plasticizers

During the formulation of edible film and coating compositions, plasticizers are frequently incorporated to reduce intermolecular interactions within biopolymers. These interactions contribute to a rigid and brittle biopolymer structure. Ideal plasticizers are biodegradable, non-volatile, and non-toxic. Two primary classifications exist based on their structural

properties: internal and external plasticizers. Internal plasticizers, introduced via co-polymerization reactions, integrate within polymer chains, creating space for enhanced polymer molecule mobility and reducing brittleness. Conversely, external plasticizers do not form chemical bonds with the biopolymer but physically interact to induce swelling. These external plasticizers are typically low-molecular-weight liquids with semi-rigid structures and high flexibility. Water molecules are a common plasticizer, particularly for protein and polysaccharide-based edible films and coatings. However, their ease of dehydration at low humidity presents a drawback. Consequently, hydrophilic plasticizers are increasingly preferred as they can mitigate water loss from the edible material by hindering dehydration.

Among various plasticizers, glycerol enjoys widespread acceptance, particularly for agar-based films due to their structural similarities. Other suitable options include carbohydrate-based plasticizers such as sugars (glucose, fructose, and sucrose) and polyols like sorbitol, glycerol, and polyethylene glycol. These food-grade plasticizers often harbor hydroxyl groups that form hydrogen bonds with the biopolymer, thereby decreasing brittleness and enhancing flexibility. Lipid-based plasticizers, encompassing waxes, fatty acids, and lecithin oils, also offer utility. Notably, the primary limitation associated with plasticizers is their potential to influence film permeability to aroma, moisture, and gases **(Walait et *al*., 2023).**

III.3.2.Additives

Additives are introduced into edible films and coatings to imbue them with specific properties such as anti-browning, antimicrobial, or antioxidant effects. These additives are also utilized to optimize rheological characteristics, intensify color, enhance flavor and aroma, and infuse nutraceuticals into the films. The inclusion of nutraceuticals and pharmaceuticals in these films facilitates their role as carriers for drug delivery. In polymer-based edible films and coatings, nanomaterials are incorporated to confer specialized properties onto packaged products that are unattainable with unmodified films. Nanomaterials seamlessly integrate these desired properties without compromising the transparency and permeability of the film **(Walait et *al*., 2023).**

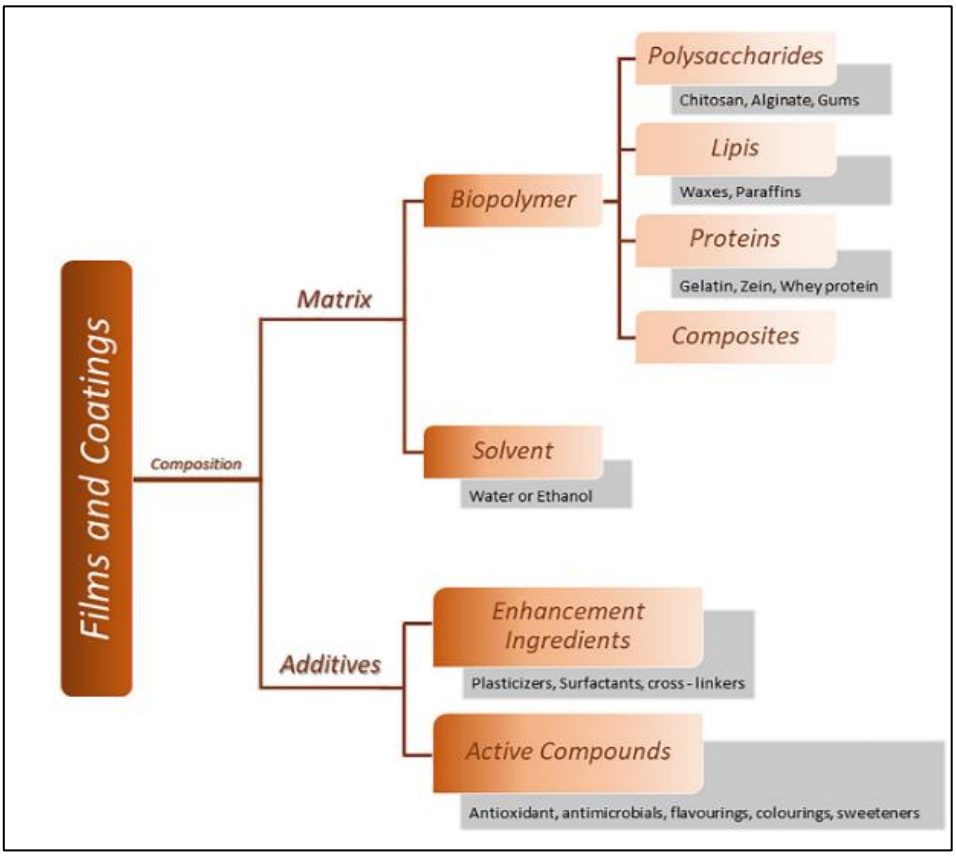


Figure 14 : Typical composition of an edible film or coating **(Walait et *al*., 2023).**

III.4. Main polymer used for biofilms preparation

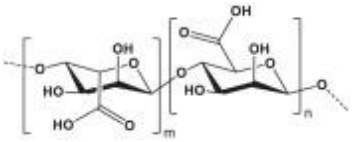
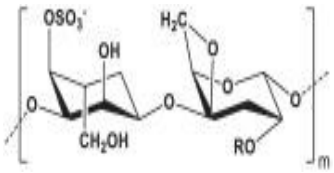
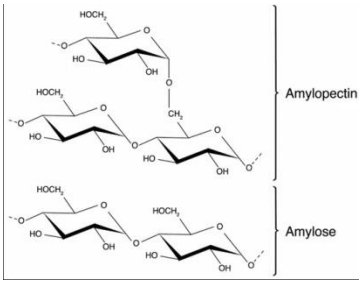
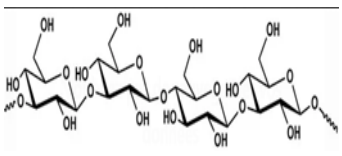
Most edible films and coatings are derived from biomacromolecules sourced from natural origins, exhibiting low toxicity levels and meeting Generally Recognized as Safe (GRAS) standards. These biomacromolecules offer enhanced processing ease owing to their biocompatible and biodegradable nature (**Liyanapathiranage et *al*., 2023).** Biopolymers like polysaccharides, proteins, and lipids are utilized in the fabrication of these edible films and coatings, serving as viable substitutes or supplements to conventional materials, thereby contributing to the reduction of traditional polymeric packaging **(Tavassoli-Kafrani et *al*., 2016).**

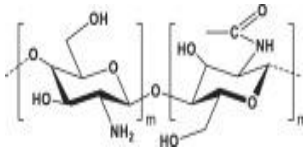
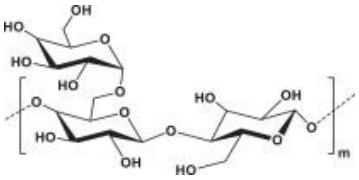
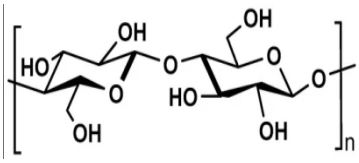
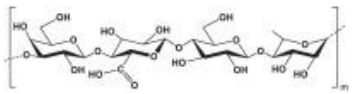
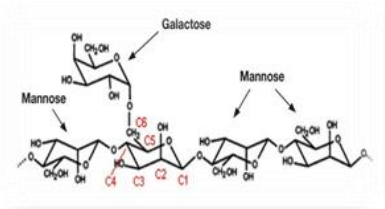
III.4.1. Polysaccharide-based biofilms and edible films

The majority of polysaccharides find widespread application in the food industry and are endorsed by the FDA, rendering them entirely appropriate, non-toxic, and secure foundational elements for fabricating bio-based materials intended for utilization in the food packaging domain. Owing to their exceptional film-forming and gel-forming characteristics, a diverse array of polysaccharides have been harnessed for fabricating thin film membranes and gels for deployment in the realms of food, pharmaceuticals, and medicine. Furthermore, recent

advancements in food packaging methodologies encompass the evolution of polysaccharide-based edible films **(Nešić et *al*., 2019).**

Table 03: Rhesus polysaccharides used in the manufacture of biofilm.

Polysaccharide	Structure	Properties	Origins
Alginate		The capacity for film formation, gelation, absence of toxicity, pH sensitivity, hydrophilic properties, biocompatibility, biodegradability, processability, and utilization of ionic crosslinking mechanisms are key characteristics in the realm of edible films and coatings.	Obtained from brown algae species (Phaeophyceae) like Laminariahyperborea, Laminariadigitata, Laminaria japonica, Ascophyllumnodosum, and Macrocystispyrifera, alginate can also be synthesized from bacterial sources such as Azotobacter and Pseudomonas.
Carrageenan		The augmentation of viscosity, gelation, stabilization, anti-neoplastic, immunomodulatory, antihyperlipidemic, and anticoagulant properties.	Red algae (Rhodophyta) represent another significant source of biomacromolecules with desirable properties for edible film and coating development. Species belonging to the genera *Chondrus*, *Eucheuma*, *Gigartina*, and *Kappaphycus* are particularly noteworthy due to their abundance and the well-characterized functionality of their extracted biopolymers.
Starch		odorless, non-toxic, tasteless, Preventing fat oxidation.	corn, tapioca, rice, cassava, and potato starch.
β-glucans		Physically stable,possessing satisfactory mechanical strength and effective gas barrier characteristics conducive to the preservation of food against oxidative degradation.	can be found in fungi, mushrooms, yeasts, algae, bacteria, and plants, mainly cereals.

Chitosan		High antibacterial efficacy, biocompatibility, biodegradability, non-cytotoxicity, hemostatic properties, rigidity, and fracture resistance are essential attributes in biomedical materials.	Derived from the chitin found in crustaceans such as lobsters, crabs, and shrimp, as well as from piscine scales and various other organisms encompassing insects and fungi.
Gum arabic		The capacity for film formation, gelation, emulsification, thickening, non-toxicity, biocompatibility, biodegradability, and antibacterial efficacy are key characteristics observed in this substance.	Originating from the exudate of Acacia Senegal.
Cellulose		Cellulose exhibits exceptional film-forming aptitude, remarkable chemical stability, and offers the advantageous characteristic of readily synthesizable derivatives.	This compound is present in the cell walls of all plant species, as well as in certain fungi, algae, marine organisms belonging to the tunicate family, various invertebrates, and select Gram-negative bacterial species.
Gellangum		the properties of gelling, flexibility, biocompatibility, biodegradability, non-toxicity, heat resistance, and acid resistance are crucial factors to consider in material design and development.	Generated through aerobic submerged fermentation of Sphingomonas elodea.
Galactomanan		Thickening and stabilizing agents, Given their significant functions in food processing and enhancing the sensory attributes and structural properties of food items.	Locust bean, guar, tara, and fenugreek are natural polysaccharidees commonly employed in the food industry due to their functional properties.

Gellangum **Gum Arabic** **Chitosan** **Carrageenan** **Alginate**	**(Ruan et *al.*, 2023).**
Cellulose **β-glucans** **Starch**	**(Nešić et *al.*, 2019).**
Galactomanan	**(Tedjani., 2023).**

III.4.2. Protein based biofilms and edible films

The functional attributes of proteins encompass their capacity to generate films and coatings. Proteins are polymers characterized by unique amino acid sequences and molecular architecture. The sequential arrangement of amino acids in a protein dictates the formation of distinct structures along the polymer chain, thereby determining the secondary, tertiary, and quaternary structures. These structures of proteins can be readily manipulated to optimize protein configuration, protein interactions, and the resulting film properties. Protein-based films and coatings are often edible or biodegradable, making them environmentally friendly and suitable for various applications **(Pérez-Gago et *al*., 2012).**

Table 04 : Proteins used in the manufacture of biofilm.

Proteins	Properties	Origins	References
Collagen	It exhibits tissue biocompatibility and non-toxicity to the majority, accompanied by well-documented structural, physical, chemical, and immunological properties.	Collagen proteins are the predominant structural components of connective tissues, including bone, dermis, tendons, cartilage, and ligaments. Notably, they comprise roughly one-third of the total body protein content in mammals, derived from animal hides.	**(Wittaya et *al*., 2012).**
Whey	The capacity for film formation, acid stability, and aeration characteristics.	Whey is a yellowish-green liquid, soluble in water, and is produced in the dairy industry.	**(Walait et *al*., 2023).**
Soy	adhesiveness, cohesiveness, dough rheology, emulsification properties, as well as water and fat absorption capacities, which collectively influence fiber formation and textural attributes.	of soybeans.	**(Chen et al.,2019). (Bourtoom et *al*., 2008).**
Casein	Biodegradability, high thermal stability, nontoxicity, the capacity to bind small molecules and ions, and the capability for micelle formation.	Casein a major milk protein.	**(Chen et *al*., 2019).**

III.4.3. Lipids based biofilms and edible films

Lipids constitute a category of organic compounds that are primarily derived from living organisms, incorporating a diverse range of sources such as plants, animals, and insects. These substances exhibit limited solubility in water but demonstrate solubility in specific organic solvents, including methanol, hexane, diethyl ether, benzene, and chloroform. To facilitate processing, lipids necessitate high temperatures.Notably, lipids are characterized by their economic viability and widespread availability in nature, leading to their extensive utilization in the production of edible films and coatings. By minimizing moisture loss and reducing packaging expenses, lipids contribute significantly to the preservation and protection of food products. Furthermore, they impart a glossy finish, thereby augmenting the visual appeal of food items **(Devi et *al*., 2024).**

Table 05: Lipids used in the manufacture of biofilm **(Liyanapathiranage et *al*., 2023).**

Lipids	Origins
Fats, shortening and Margarine	Various types of fats and oils derived from animals, plants, and seeds, including butter from dairy sources, lard, sunflower oil, mustard oil, olive oil, almond oil, peanut oil, coconut oil, palm oil, cocoa butter, as well as fractionated, concentrated, or reconstituted oils and fats. Additionally, mono-, di-, and tri-glycerides, hydrogenated or trans-esterified oils, such as those found in margarine and shortening, are also included.
Waxes	Natural waxes derived from animals, insects, and plants include beeswax, carnauba wax, candelilla wax, genuine rice bran wax, and laurel wax. Synthetic waxes, on the other hand, encompass paraffin wax, mineral wax, microcrystalline wax, and oxidized/non-oxidized polyethylene wax.

Natural resins	Gum Arabic, Benzoin, Chicle, Guarana, Myrrh, Frankincense, Opoponax, Sandarac.
Essential oils and liquorices	variety of natural sources : Flowers(Rose oil), Vegetables (Ginger oil), Animals , Fruits (Citrus oil, Mint oil).

III.5. Application

Edible films and coatings refer to thin materials utilized to envelop or cover a product, enhancing its shelf life while being consumable alongside the product. They serve to enhance the quality of perishables like fruits and vegetables by safeguarding them against alterations caused by physical, chemical, and microbiological factors such as moisture loss, enzymatic browning, and lipid oxidation. Edible coatings, typically in liquid form, are administered by immersing the raw material in the solution, while edible films, resembling solid sheets, are applied by encasing the product **(Kocira et *al*., 2021).**

III.5.1.Application to fruits and vegetables

Previous Research findings indicate that the application of a 10% gum arabic coating on tomato fruit resulted in a notable retardation of weight alteration, firmness, titratable acidity, soluble solids concentration, and color evolution over the course of storage at 20°C, relative to untreated control specimens. Furthermore, sensory assessments underscored that the utilization of a 10% gum arabic coating sustained the holistic quality of the tomato fruit throughout the storage period **(Ali et *al*., 2010).**

In this study, blackberries stored at 40°C for 20 days were treated with an edible and biodegradable film based on starch and nystose to extend their shelf life. The experiment involved three categories of samples: uncoated control fruit, fruit coated with starch, and fruit coated with an edible starch-nystose coating. The findings indicated that the fruit samples coated with starch and starch-nystose exhibited beneficial effects in delaying the increase in pH and preserving firmness and anthocyanin content. Sensory analysis revealed that the coated samples were as appealing to buyers as the control samples, with no significant visual modifications. The application of edible coatings may contribute to improving the fruit's external appearance and reducing fungal growth by slowing down respiration and senescence **(Miteluț et *al*., 2021).**

III.5.2.Application to cheese

Cheese represents a consumable dairy product susceptible to surface contamination by deleterious microorganisms, thereby leading to spoilage and the development of undesirable flavors. Moreover, certain cheese varieties are prone to experiencing adverse organoleptic changes due to heightened moisture loss. Utilizing prepared whey protein films for safeguarding Cheddar cheese revealed that product characteristics and sensory attributes remained unaltered by the application of coatings. The implementation of an edible coating comprising whey proteins infused with antimicrobial agents serves as an efficacious strategy for impeding the proliferation of harmful microorganisms and extending the product's shelf life. Films composed of whey protein isolates, augmented with nisin (50 IU/mL), malic acid (3%), sorbitol (1.5%), and natamycin (0.002 g/mL), exhibited inhibitory effects on the surfaces of cheese against various microorganisms including *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Penicillium commune*, *Penicillium chrysogenum*, and *Yarrowia lipolytica*. Furthermore, Ricotta cheese coated with chitosan/whey proteins edible film and stored at 4°C for 30 days demonstrated a noteworthy decrease in mesophilic and psychrotrophic counts compared to untreated samples. Employing edible antimicrobial coatings for cheese offers a viable alternative to conventional commercial coatings, particularly in terms of enhancing moisture retention and serving as a barrier against weight loss **(Kandasamy et *al*., 2021).**

III.5.3.Application on cereal bars

In another study, synbiotic-supplemented whey protein or alginate coatings were used for cereal bars and it was revealed that physicochemical properties (aw, moisture content, color and texture) were not affected during storage. Appearance, color, odor, flavor, texture, crunchiness, and adhesiveness were analyzed using a 9-point hedonic scale by 5-point ratings, where 1 and 2 corresponded to “too weak” (TW), 3 to “just about-right” (JAR), and 4 and 5 to “too strong” (TS) **(Seyedzade Hashemi et *al*., 2022).**

III.5.4.Application to meat and fish

The incorporation of rosehip extracts into a rye starch matrix yielded cast edible composite films subsequently employed for chicken breast packaging. This modification resulted in enhanced flexibility, optical properties, and antioxidant activity within the films. Notably, the films containing 1.0% extract exhibited the highest ABTS and DPPH radical scavenging activities, reaching 96.87% and 80.22%, respectively. Additionally, chicken breasts packaged with these films displayed demonstrably lower peroxide and thiobarbituric acid reactive

substance (TBARS) values compared to those packaged with the unmodified rye starch film or stored without packaging altogether. These findings suggest the efficacy of the edible composite films in effectively inhibiting lipid oxidation and extending the shelf life of chicken breast. Similarly, the inclusion of maqui berry extract, carvacrol, and chitosan within starch-based edible composites (encompassing films and coatings) has been shown to retard lipid oxidation in various foodstuffs such as fish and ham, while concurrently inhibiting the proliferation of foodborne pathogens and ultimately extending the products' shelf life. Furthermore, Lycium ruthenicum Murr anthocyanins were successfully integrated with cassava starch to fabricate a novel freshness indicator film exhibiting both intelligent pH sensitivity and edibility, ideal for pork packaging. This composite film demonstrated improved barrier properties, tensile strength, and antioxidant activity due to hydrogen bond interactions between the anthocyanins and starch chains. Notably, the film facilitated real-time and visual monitoring of pork freshness through a color change response reflecting the quality of the pork during storage **(Zhao et *al*., 2021).**

III.6. Basic functions and methods for preparing edible films and coatings

III.6.1. Basic functions edible films and coatings

In the realm of produce preservation, edible films and coatings serve as pivotal protective barriers, offering a regulated atmosphere conducive to the longevity of fruits and vegetables. Figure 15 encapsulates underlying the application of edible films and coatings in the preservation of such produce **(Liyanapathiranage et *al*., 2023).**

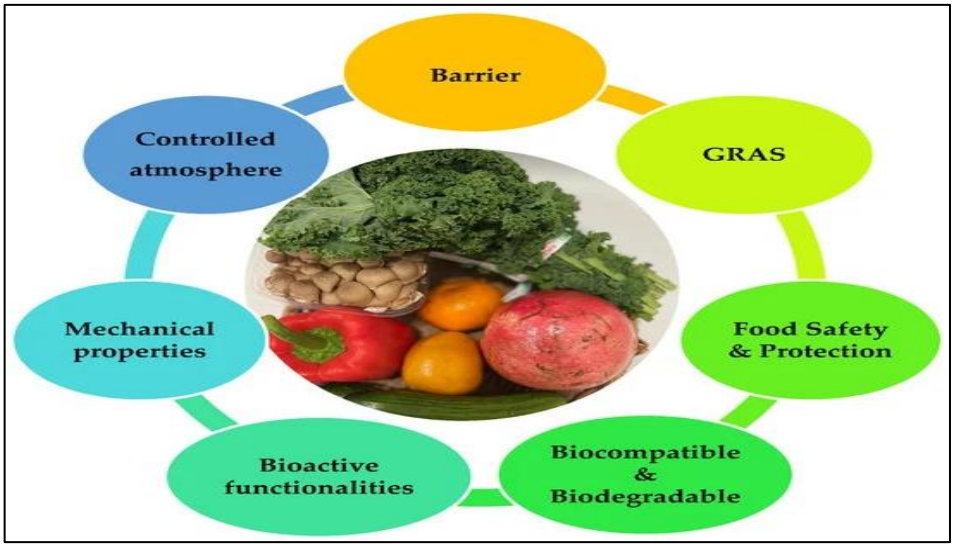


Figure 15: The basic functions of edible films and coatings in preserving vegetables and fruits **(Liyanapathiranage et *al*., 2023)** .

Edible films and coatings offer a multitude of functionalities, including:

- **Mechanical protection:** Shielding against physical damage incurred during transport, handling, and mitigating the effects of UV radiation.
- **Selective barrier properties**
    - **Moisture control:** Minimizing water vapor permeability to retard dehydration.
    - **Gas management:** Regulating the permeation of oxygen and carbon dioxide through the film.
    - **Aroma and solvent barrier:** Impeding the passage of volatile organic compounds (VOCs) such as aromas, solvents, and other additives or pigments.
- **Shelf life extension:** Enhancing the shelf life of produce.
- **Bioactivity:** Exhibiting antimicrobial and antifungal properties, potentially functioning as probiotics.
- **Biodegradability:** Decomposing readily by natural processes.
- **Structural integrity:** Possessing a melting point above 40°C without degradation, exhibiting water resistance, allowing for easy emulsification, and maintaining a non-sticky or non-tacky surface while facilitating efficient drying.
- **Preservation of food quality:** Minimally affecting the texture, flavor, or color of the food product.
- **Economic and safe composition:** Formulated from cost-effective, relatively abundant, and Generally Recognized As Safe (GRAS) materials, ensuring consumer safety **(Liyanapathiranage et *al.*, 2023).**

III.6.2. Common methods for preparing edible films and coatings

In the fabrication of edible films and coatings, the choice of raw materials and the configuration of layers exert a considerable influence. Moreover, the intrinsic properties of the edible film can dictate its overall mechanical strength, solubility, interfacial behavior, visual aesthetics, gustatory perception, and oral texture. Edible films are predominantly fabricated in the form of sheets or thin wraps, subsequently employed to envelop food items intact. The fabrication processes typically employed for edible films and coatings include melt extrusion and solvent casting technics **(Liyanapathiranage et *al.*, 2023).**

III.6.2.1. Spraying

This approach leverages the phenomenon of droplet deposition onto the designated substrate, resulting in the creation of a semi-permeable membrane composed of the coating material on the food item. The physicochemical attributes such as density, viscosity, and surface tension of the biopolymer substance, in conjunction with the operational parameters (temperature and air pressure), typically dictate the characteristics and attributes of the coating. The primary benefit of employing this methodology lies in its cost-effectiveness, which is facilitated by its scalability to cover extensive surface areas, precise temperature regulation, and its ability to prevent any form of contamination. Furthermore, the coating generated through the spraying process is typically less than 20 μm in thickness, allowing for controlled coating thickness **(Walait et *al*., 2023).**

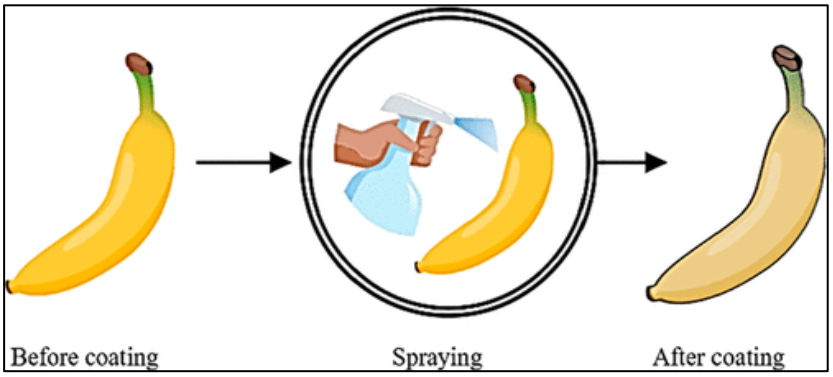


Figure 16: Application of paint by dispersion method **(Matloob et *al*., 2023).**

III.6.2.2. Dipping

Dipping is a technic in which the product is immersed in the coating solution and it occurs in four steps including two immersion cycles. In the first step the food product is immersed or dipped in the biopolymer solution. In second step the biopolymer solution is placed in the cross linking bath and the material to be coated is placed in that bath. The solution is drained at regular intervals or after each stage. Contrary to the spraying method this method generates a thick coating on the food product. This property is controlled by determining the density, viscosity and surface tension of the biopolymer solution used. This technic is generally applied on the food that is minimally processed **(Walait et *al*., 2023).**

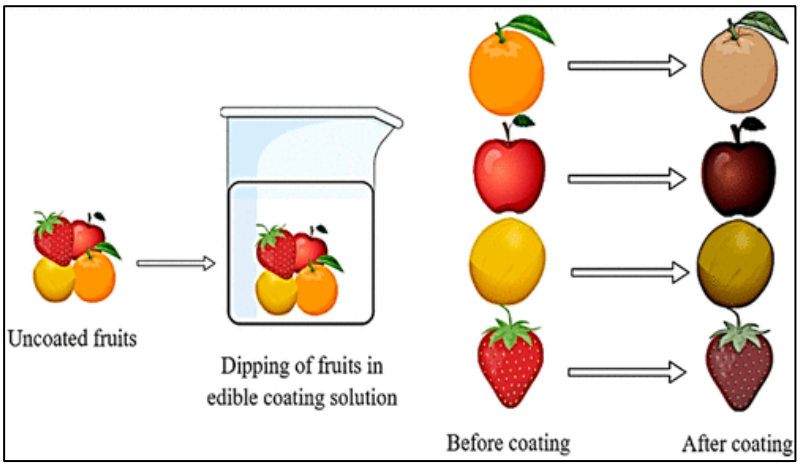


Figure 17 : Immersion paint application**(Matloob et *al*., 2023).**

III.6.2.3. Vacuum impregnation

Vitamins and minerals are essential micronutrients that the body requires for various physiological processes. To fortify food products with these vital elements, a technic known as vacuum impregnation is often employed. This method is particularly well-suited for fruits and vegetables with a porous outer layer, which allows air pockets within these pores to be replaced with the desired solutes. As a result, vacuum impregnation can facilitate the formation of thicker and more robust coating films compared to traditional dipping methods. The overall process shares similarities with dipping, but with a crucial distinction: vacuum chambers are utilized instead of conventional dipping tanks. Following application of the coating solution, the food product remains submerged for a designated period. Subsequently, the process concludes with atmospheric restoration, a critical step that ensures uniform distribution of the coating material across the food item. Both the pressure within the vacuum chamber and the meticulous control of atmospheric restoration are paramount for achieving this even application **(Walait et *al*., 2023).**

Experimental part

CHAPTER IV

Materials and methods

IV. Materials and methods

This study focused on the investigation of the water-soluble polysaccharides extracted from *Retama raetum* seeds harvested in the El-Oued province (septentrional Algerian Sahara). And the application of this molecule for food preservation and packaging. This research contributes to expanding the repertoire of polymer that can be utilized as sustainable alternatives to plastic food packaging.

IV.1. Plant Harvesting

The *Retama raetum* was harvested in their natural habitat, away from areas affected by human activity, to avoid any disturbance. Harvests were conducted in October 2023. The plant sample was collected in the El Magrane region GPS: 33°74'44.9" N and 6°91'02.10" E in the El-Oued Wilaya (Algeria). The choice of plant is based on the search for a suitable source of polysaccharides. The selection of the plant part depends on their chemical composition, polysaccharides generally concentrated in the seeds.

Figure 18: *Retama raetam* **(Original picture., 2024)**.

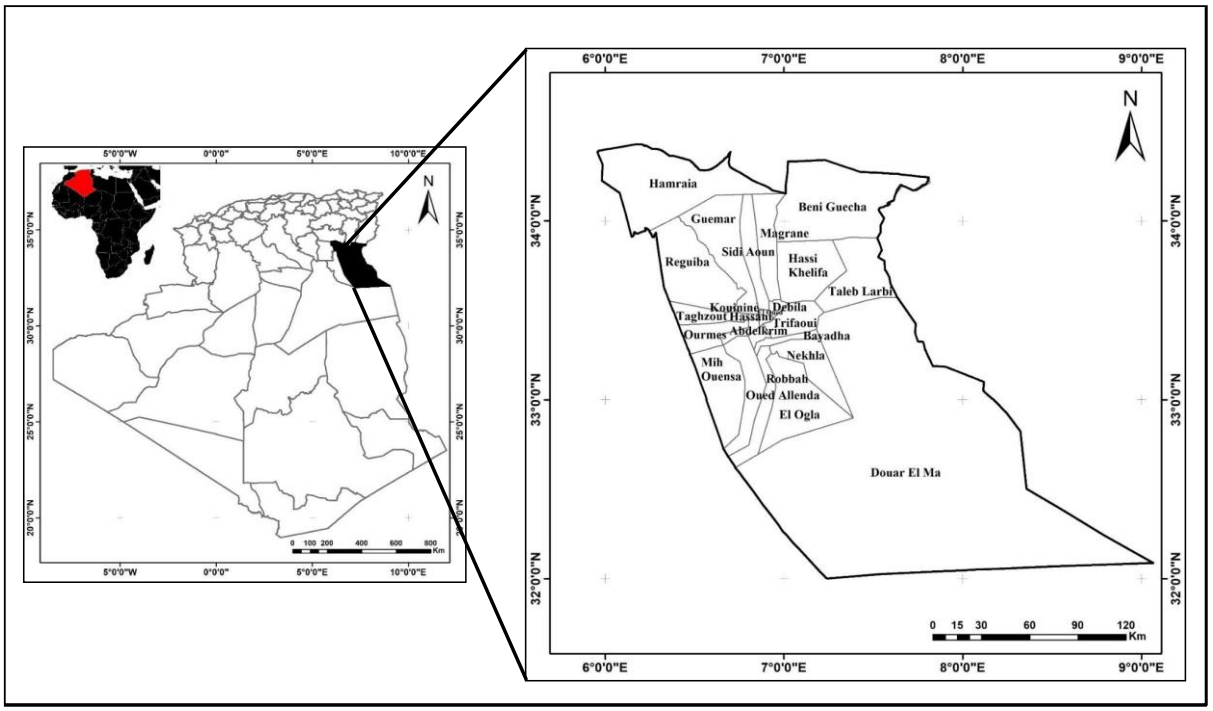


Figure 19: El Magrane region GPS: 33°74'44.9" N et 6°91'02.10" E **(Original picture., 2024)**.

Figure 20 : *Retama reatam seeds* ***(original picture., 2024)***.

Figure 21: *Retama reatam* crushed seeds **(Original picture ., 2024)**.

IV.2. Identification of plant

The plant sample was identified by the expertise of Dr. Slimani Noureddine.

IV.3. Extraction of the water-soluble Polysaccharides

Polyssacharids were extracted from dried *Retama raetam* plant seeds using various methods. Initially, 60 g of *Retama raetam* seed powder were suspended in 700 mL of distilled water. The mixture was stirred for 2 h at 65°C and 500 revolutions per minute (rpm), resulting in gel formation. Subsequently, the mixture was cooled to 4°C and concentrated using centrifugation at 4000 rpm to separate the gel. The precipitates were recovered, and extraction was carried out 3 times using water at 65°C for 2 hours each time. The upper layers were collected and concentrated using a rotary evaporator under low pressure at 65°C. The concentrate was then cooled to 4°C, and polysaccharides were precipitated using absolute ethanol overnight. The final precipitates were washed with acetone (500 mL) and dried for 24 hours at 45°C, followed by grinding into a fine white powder known as PSRR **(Chouana., 2017).**

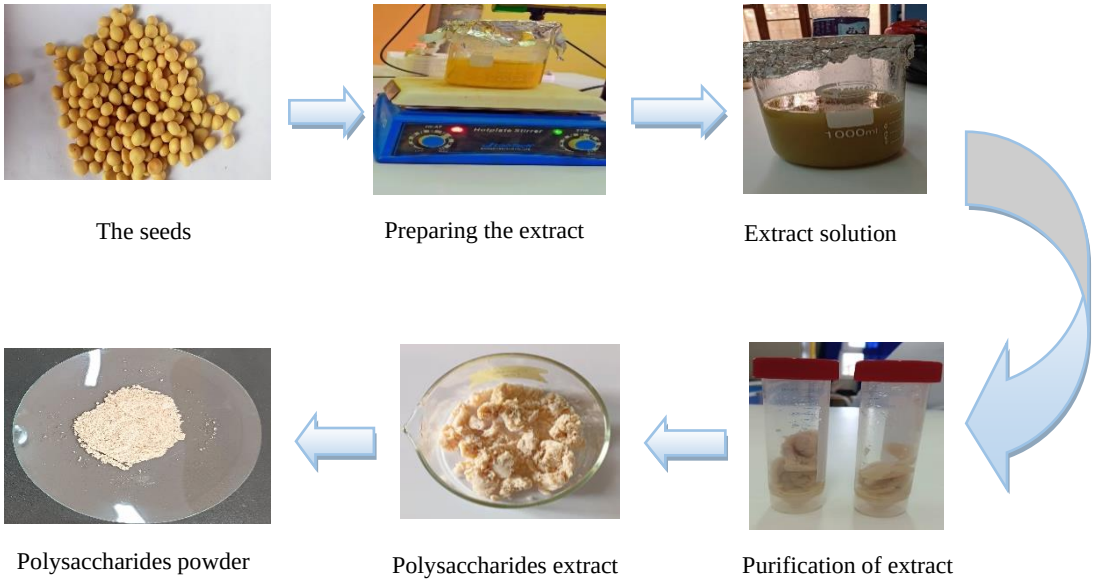


Figure 22: Extraction protocol for water-soluble polysaccharides from Retama raetam

IV.3.1. Purification of the water-soluble Polysaccharides

The final pellet was washed 3 times with acetone (500 mL). The polysaccharide was dried at 45 °C for 24 hours in open air and then ground into a fine brown powder using a mortar.

IV.3.2. PSRR mining yield

The output of the yield loss ratio (PSRR) represents the ratio of the final extracted weight compared to the initial material weight relatively, expressed as a percentage. This ratio is calculated using the following mathematical formula **(Guemmane & Kerfel., 2016) :**

$$R = \frac{P_1}{P_0} \times 100$$

R : Extraction yields

P_1 : Final weight

P_0 : Initial weight

IV.4. Mesurement of the conductivity:

Principe: Using electrical conductivity measurement technic, the chemical nature of polysaccharides can be determined, such as whether they are negatively charged, positively charged, or uncharged. This is achieved by measuring conductivity levels using a specialized

conductivity measuring device, which contributes to accurately categorizing these classes scientifically **(Armane &Arkam., 2016).**

Materials and Reagents: Acetone (Sigma Aldrich ACS reagent, ≥99.5%), distilled water,ethanol (Sigma Aldrich02851), extract polysaccharides (PSRR), Conductivity meter, Centrifuge(DM0412).

Procedure: A conductivity measurement device was utilized to assess the conductivity of ethanol and acetone, thus determining the residual salt quantity in the sample. Subsequently, the final precipitate was washed with acetone (500 mL) 3 times using a centifugueuse device for 15 min at 4000 revolutions. The polysaccharide was then dried at a temperature of 45°C for 24 hours in an outdoor environment.

IV.5. Quantification of the chemical composition of the Polysaccharide extract

The quantification of total sugars, neutral sugars, proteins, and polyphenols in the polysaccharide fraction extract was determined through colorimetric methods.

Preparation of polysaccharide extract solutions to formulate the polysaccharide extract solutions, 10 mg of each crude polysaccharide extract was dissolved in 100 mL of distilled water and stored at 4°C.

IV.5.1. Quantification of Total Sugar

IV.5.1.1. Quantification of Total Sugar Using Dubois Method

Principe: The amount of total sugars is assessed using the colorimetric assay method by (**Dubois et *al*., 1956).** Glycosidic linkages are hydrolyzed in the presence of concentrated sulfuric acid, leading to the dehydration of osidic units and the formation of furfuric compounds. These compounds react with phenol through condensation, resulting in orange-yellow colored compounds that absorb at a wavelength of 492 nm.

Materials and Reagents: Phenol (Sigma-Aldrich, PL037), 5% (w/v) aqueous phenol solution, 80% sulfuric acid (Honeywell, 30743), glucose (Sigma-Aldrich, G8270), 100 µg/mL aqueous glucose solution with necessary dilutions, distilled water, water bath (Julabo, TW12), spectrophotometer (SHIMADZU1800).

Procedure:

Preparation of a 5% phenol solution: Dissolve 1g of phenol in 20 mL of distilled water.

Preparation of the primary glucose solution at 100 µg/mL: Dissolve 10 mg of glucose in 100 mL of distilled water.

In glass assay tubes, dispense 200 µL of the sample and immerse the tubes in an ice bath. Subsequently, introduce 200 µL of a 5% aqueous solution of phenol and homogenize. Carefully add 1 mL of sulfuric acid H2SO4 (80%) at 90°C for 30 min, followed by a 30 min incubation at room tmperature in the dark. Measure the absorbance at λ = 490 nm.

IV.5.1.2. Quantification of Total Sugar Using refractometer Method

Principe: The sample is placed in contact with one face of a prism, where the refractive index of the prism is higher than that of the sample. The system is illuminated by a set of low-angle incident rays passing through the contact surface. Upon refraction, these rays create a bright zone and a dark zone, with the location of their boundary depending on the refractive index. **(Dahamna & Chergui., 2020).**

Materials and Reagents: glucose (Sigma-Aldrich, G8270), 100 µg/mL aqueous glucose solution with necessary dilutions, distilled water ,refractometer.

Procedure: We prepared various solutions of glucose at different concentrations (0, 0.3125, 0.625, 1.25, 2.5 mg/mL) by dissolving 10 mg of glucose in 100 mL of distilled water. Additionally, a solution of PSRR compound was prepared at a concentration of 2.5 mg/mL in 10 mL of distilled water. These solutions were examined using a refractometer, where the refractive index of each solution was measured after wiping the instrument's prism with absorption paper and calibrating it with distilled water (refractive index = 1.33). Subsequently, drops of the samples were placed on the prism in the instrument and sealed to measure the refractive index in the device's lens **(Dahamna & Chergui., 2020).**

IV.5.2. Quantification of neutral sugars

Principe: The determination of the quantity of constituent neutral sugars relies on the colorimetric assay method outlined by (**Monsigny et *al.*,1988)**. This method involves a quantitative hydrolysis of glycosidic linkages in polysaccharides in the presence of concentrated and hot sulfuric acid. This hydrolysis releases osidic units, which subsequently undergo dehydration to form furfuric derivatives. These furfuric derivatives are capable of condensing with resorcinol in an acidic medium, yielding a brown-orange complex. It is noteworthy that this colorimetric assay method is highly sensitive and enables precise measurement of the amount of constituent neutral sugars in a given sample.

Materials and reagents: Resorcinol (Sigma-Aldrich, 398047), aqueous solution of resorcinol at 0.6% (w/v), 80% sulfuric acid (Honeywell, 30743),glucose (Sigma-Aldrich, G8270), aqueous solution of glucose (100 µg/mL with necessary dilutions),distilled water, water bath (Julabo, TW12) spectrophotometer **(**SHIMADZU1800).

Procedure:

Preparation of 0.6% resorcinol solution: To prepare the 0.6% resorcinol solution, dissolve 6 mg of resorcinol in 1 mL of Milli-Q water. This solution can then be stored at 4°C, where it remains stable for 4 weeks.

Preparation of 100 µg/mL glucose stock solution: To prepare the 100 µg/mL glucose stock solution, dissolve 10 mg of glucose in 100 mL of distilled water. This solution can be used to prepare diluted glucose solutions for assays.

Glucose quantification: To assay glucose, 200 µL of the sample is dispensed into a assay tube, followed by 200 µL of the resorcinol solution and 1 mL of H2SO4. The mixture is stirred, then incubated in a dry water bath at 80°C for 30 minutes. After returning to room temperature for 30 min in darkness, absorbance is measured at 450 nm using a spectrophotometer. The quantity of glucose in the sample can be determined by comparing the sample's absorbance to that of a series of glucose standards with known concentrations.

IV.5.3.Protein assays

IV.5.3.1.Protein assays using the Bradford Method

Principe: The protein quantity is determined through the colorimetric Bradford method **(1976)**, based on the formation of a complex between Coomassie G-250 blue and proteins in solution. Coomassie blue primarily binds to the arginine, tryptophan, tyrosine, histidine, and phenylalanine residues of proteins **(Chebrou et *al*., 2022).**

Materials and reagents: Bradford reagent,Bovine Serum Albumin (BSA) (Sigma-Aldrich, A2153). Aqueous BSA solution (100 µg/mL with necessary dilutions),distilled water, vortex,spectrophotometer (SHIMADZU1800).

Procedure:

Bradford reagent preparation: Dissolve 100 mg of coomassie blue in 50 mL of ethanol (95%). Stir the mixture for 2 h with a stirrer in the absence of light. Then add 100 mL of orthophosphoric acid (85%). Complete the volume to 1L with distilled water. Filter the obtained solution with filter paper. This reagent is stable for 2 weeks at 4°C.

Preparation of 0.01% BSA stock solution: Prepare by dissolving 10 mg of BSA in 100 mL of distilled water.

Dispense 500 µL of the sample into assay tubes, followed by 500 µL of Bradford reagent. Vortex the tubes for 5 s, allow them to stand at room temperature and in darkness for 30 min, and measure the absorbance at 595 nm.

IV.5.3.2.Protein assays using Warburg and Christian Method

Principe: Most proteins exhibit strong ultraviolet absorption at 280 nm, primarily attributed to the presence of 2 key amino acids, tyrosine and tryptophan. Due to variations in the content of these amino acids among different enzymes, albeit within reasonable limits, the 280 nm absorption peak has been widely adopted as a rapid and sensitive method for protein concentration measurement. However, it is notable that nucleic acids, which may be present in enzyme preparations, show significant absorption in the ultraviolet region at 280 nm. Interestingly, nucleic acids exhibit even stronger absorption at 260 nm compared to 280 nm, whereas the opposite is observed with proteins. Warburg and Christian leveraged this phenomenon in their research endeavors **(Warburg and Christian., 1941).**

Materials and reagents: Sample PSRR, Spectrophotometer (SHIMADZU1800).

Procedure: We dissolved 1.5 mg of polysaccharide extract in 1 ml of distilled water. After thorough mixing, 900 µL of the solution were taken and diluted with 100 µL of distilled water. Subsequently, absorbance was measured using a spectrophotometer at wavelengths of 260 nm and 280 nm to assess the protein content. These wavelengths were selected due to their alignment with the maximum absorption of nucleic acids and proteins, making them suitable for evaluating the composition of the mentioned compound.Protein concentration is determined following the Warburg and Christian **(1941)** equation:

Protein concentration (mg/mL) = (1.55 × OD at 280) – (0.76 × OD at 260)

IV.5.4.Assay of total phenolic compounds

Principe: The quantification of phenolic compounds is determined using the colorimetric assay method developed by (**Singleton et *al.*, 1999)**. The Folin-Ciocalteau reagent contains phosphomolybdate and sodium tungstate, which oxidize polyphenols, leading to the formation of tungsten and molybdenum oxides (blue coloration). The reduction of these metal ions causes an increase in absorbance measured at 760 nm, enabling the quantification of phenolic compound concentration in the sample.

Materials and Reagents: The Folin-Ciocalteu reagent (Sigma-Aldrich, F9252), sodium carbonate (Sigma-Aldrich, S7795), aqueous solution of 10% sodium carbonate, gallic acid (Sigma-Aldrich, G7384), aqueous solution of gallic acid (100 µg/mL with appropriate dilutions),ultrapure water, spectrophotometer (SHIMADZU 1800).

Procedure:

Prepare the Folin reagent 1/10: Dilute the Folin reagent 10-fold with distilled water.

Prepare a 20% sodium carbonate solution: Dissolve 20 g of sodium carbonate in 100 mL of distilled water.

Prepare the stock solution of gallic acid at 0.1%: Dissolve 100 mg of gallic acid in 100 mL of distilled water.

In the assay tubes, pipette 100 µL of the sample, add 500 µL of Folin reagent, agitate, and let it stand for 3 min. Subsequently, add 2 mL of sodium carbonate solution, mix for 10 s, and allow the mixture to rest in the dark at room temperature for 1h or until a blue color develops. Measure the optical density at 765 nm.

IV.6. Structural characterization of the polysaccharidee extracts

IV.6.1. UV-Vis analysis of the polysaccharidee extracts

Principe: The purity of polysaccharidees needs to be assessed following their extraction. Nucleic acids and proteins strongly absorb UV light within the wavelength range of 260 nm to 280 nm **(Pan and Mei, 2010; Zhbankov, Andrianova, and Marchewka, 1997).** Therefore, it is crucial to measure the absorbance of the samples at these wavelengths to estimate the potential presence of these contaminants.

Materials and reagents: Samples (powder) for analysis, spectrophotometer (SHIMADZU1800).

procedure: The optical density (OD) measurement technic was applied using a visible ultraviolet (UV) spectrophotometer in a quartz cuvette to assess the purity of carbohydrate extracts and detect potential contamination by proteins and nucleic acids. Measurements were taken at wavelengths of 260 nm and 280 nm due to their alignment with the maximum absorption of nucleic acids and proteins. 1ml of each carbohydrate extract (concentration of 5 mg/ml) was added to 1 ml of the control solution. Subsequently, absorbance was measured at the specified wavelengths to identify contaminants and evaluate sample purity **(Pan and Mei, 2010; Zhbankov, Andrianova, and Marchewka, 1997).**

IV.6.2. Infrared Spectra (FT-IR)

Principe: Fourier Transform Infrared Spectroscopy (FT-IR) analysis is a fundamental technic in organic chemistry and biology sciences. This method involves identifying functional groups in organic compounds by measuring the absorption of chemical bonds in the infrared range at specific wavelengths. Thanks to this technology, components of samples such as OH,

CH, CC groups, among others, can be precisely determined, aiding in the understanding of molecular structure and composition.

The wavelength range used in infrared analysis spans from 4000 cm-1 to 400 cm-1, encompassing molecular transitions associated with vibration and rotation. This range reflects the reactions of organic molecules in living organisms, making it particularly suitable for analyzing sugars and other bioactive compounds. This method holds special significance in identifying and analyzing biological compounds, contributing to the understanding of biological and chemical processes within living systems **(Tedjani A., 2023).**

Materials and reagents: Samples (powder) for analysis, FT-IR instrument (Nicolet iS5, Thermo Fisher Scientific).

procedure: Infrared spectroscopy was employed for the identification of various sugar compounds. To achieve this, infrared spectra were recorded for all samples using an infrared spectroscopic scale within the wavelength range of 400 to 4000 cm^-1 **.**

IV.6.3. X-ray Diffraction (XRD)

Principe: The powder X-ray diffraction characterization method provides insights into the crystalline structure of materials, including their texture and degree of crystallinity. It relies on the interaction between an electromagnetic wave, such as X-rays, and the periodic lattice of crystallized matter. This technic is commonly used for characterizing solids in powder form. The incident X-ray beam is directed at a set of randomly oriented crystallites, numerous enough to represent all orientations. This technic is particularly useful for determining the crystalline structure of complex materials like polymers, ceramics, and minerals **(Sahli., 2015)**.

Materials and reagents: Samples (powder) for analysis, X-ray diffractometer instrument (Panalytical, model Empyrem).

Procedure: X-ray diffraction measurements were performed on a Panalytical Empyrem diffractometer, utilizing CuKα radiation (wavelength λ = 1.54056 Å) at a voltage of 40 kV and a current of 40 mA. The samples were analyzed in Bragg-Brentano HD mode with an angular range of 3° to 70°, using steps of 0.1° and a measurement time of 60 seconds per step. The data were normalized and plotted to generate diffractograms. The crystallinity index (CI) of the polysaccharides and the fresh sample was calculated using Segal, Creely, Martin, and Conrad's equation **(1959).** This method allows the evaluation of the amount of crystalline material present in the analyzed samples.

IV.6.4.Scanning microscopic observation (SEM)

Principe: The scanning electron microscope (SEM) enables the study of the surface of massive samples by analyzing their interaction with low-energy primary electrons, thereby limiting their penetration to a small area. Within this area, electrons lose some of their energy and excite other electrons, known as low-energy secondary electrons, which allow for three-dimensional surface observation. When primary electrons have high energy, more electrons can be removed, termed backscattered electrons, possessing high energy and providing information about sample density. This density corresponds to a gradation of grayscale on the screen, with higher light intensity in denser areas, i.e., where heavier elements are present. The apparatus comprises an electron column producing a beam, a chamber for sample insertion, and an imaging system constructed from signals of accelerated electrons via a tungsten filament **(Al mahdi., 2006).**

Materials and reagents: Samples (powder) for analysis , Scanning Electron Microscope (SEM).

Procedure: Scanning Electron Microscopy (SEM) is a microscopic analysis technic that relies on electron-sample interactions, enabling the production of high-resolution images of the sample surface. This is achieved by directing a beam of electrons towards the sample, where electrons interact with the sample's surface layer, resulting in brighter and darker regions. This aids in studying the structural details and surface properties of the sample with high precision **(Haddadi., 2015).**

The studied samples were prepared using Scanning Electron Microscopy by fracturing them after immersion in liquid nitrogen. This helps preserve the sample's structure and fundamental properties before conducting detailed analysis using Scanning Electron Microscopy **(Elkhaoulani et *al.*, 2013)**.

IV.7. Preparation of biofilms

Principe: The preparation of biofilms necessitates the utilization of a polymer capable of forming a cohesive and homogeneous matrix. In this instance, we opted for natural polymers such as galactomannan extracted from legumes due to their favorable properties in biofilm formation. To enhance the flexibility of these biofilms, conventional plasticizers are employed. Throughout our study, we eschewed the use of additives aimed at achieving healthy biofilms devoid of hazardous substances **(Al mahdi., 2006).**

Materials and reagents: Sugar extract (PSRR), distilled water, glycerol(CAS56815), Crystallizer, (CNP004), agitator (Hotplate stirrer DAIHAN LABTECH CO.,LTD)

Procedure: The dispersion systems were prepared using the methodology outlined by (**Cerqueira et *al*., 2011)** with minor modifications. Precise amounts of sugar extract (concentrations of 1% and 1.5%) were weighed and suspended in distilled water under stirring for one hour at 25°C. Subsequently, 40% glycerol was added, and the resulting solutions for biofilm formation were homogenized under stirring at 70°C for 30 min until achieving homogeneity. The biofilm-forming solutions were then degassed under vacuum to remove as many air bubbles and dissolved air as possible. Following this, 28 ml of the filmogenic solution was poured into a crystallization device at 70°C to prevent premature crystallization at lower temperatures, before being dried at 35°C for 16h **(Martins et *al*., 2012) .**

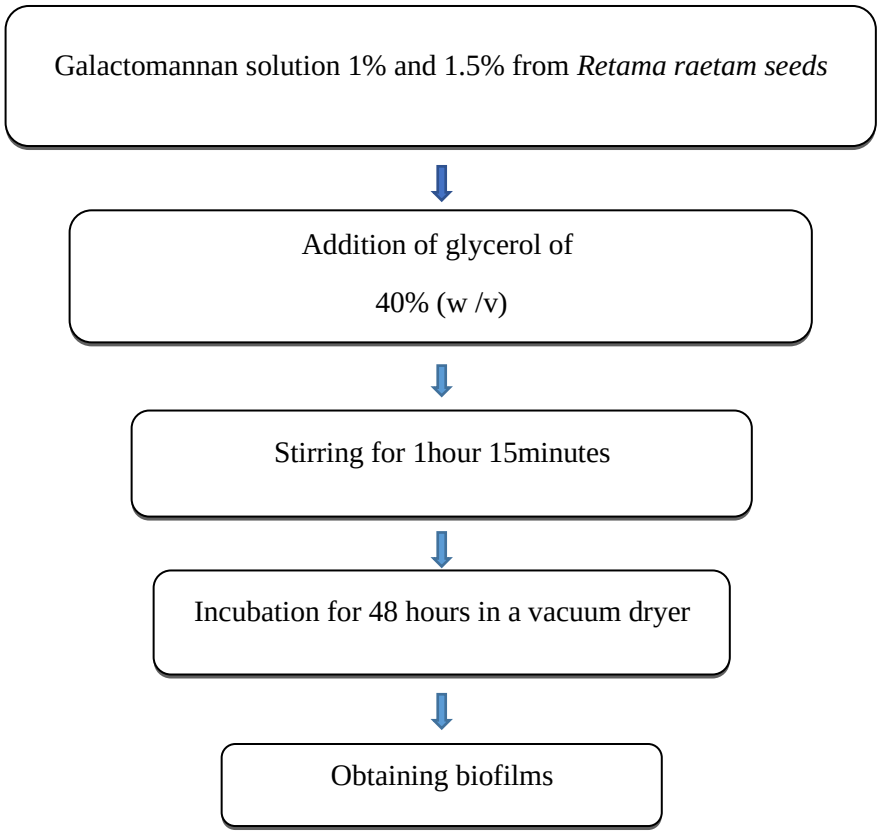


Figure 23: Biofilm preparation protocol **(Guemmane & Kerfel., 2016).**

IV.8. Study of the physical characteristics of biofilms

IV.8.1. Biofilm thickness

The thickness of biodegradable biofilms was determined using a manual micrometer (Caliper) based on the average of at least 4 random measurements conducted on each biofilm **(Nur Hanani et *al*., 2013)**.

IV.8.2. Opacity

The opacity of the biofilms was determined by measuring the optical density of rectangular pieces measuring 1×4 cm at a wavelength of 500 nm. Subsequently, the opacity was calculated using the following equation as described by (**Gontard et *al*., 1994).**

IV.8.3. Humidity level

In general terms, humidity encompasses all substances that evaporate upon heating, resulting in a sample mass loss. The moisture content of biofilms, often mistaken for their water content, was determined by drying small pieces of previously weighed biofilms in an oven at 110 °C for 6h **(Belibi et *al*., 2014)**. Weight loss is measured using a balance and interpreted as a moisture content. Consequently, this concept of humidity extends beyond water to include other mass losses such as organic solvents, alcohols, fats, oils, aromatic components, and evaporated decomposition and combustion products.

IV.8.4. Solubility of biofilms in water

The solubility of biofilms prepared in water was assessed following the methodology outlined by **(Rhim et *al*., 2005)**. Samples were randomly selected from the biofilms and initially dried in an oven at 110°C for 6h to determine their initial mass. Subsequently, each sample was placed in cups containing 40 ml of distilled water, covered with Parafilm, and stored at room temperature for 24 h. The mass of undissolved biofilm was determined by removing the biofilm pieces from the cups, rinsing them with distilled water, and drying them again in the oven (110 °C, 6 h).

IV.8.5. The angle of contact with water

This experiment was conducted following the methodology outlined by (**Gharibi et *al*., 2018) ,** (4 µL) of distilled water were deposited into the biofilm surface. The contact angle with water was measured using an angle gauge according to the official definition, where the angle is defined as the angle between the baseline and the tangent line at the point of contact between the water droplet and the biofilm surface. 05 measurements were recorded at different locations on each biofilm surface, enhancing result accuracy and contributing to the scientific study's reliability**.**

IV.9.Evaluation of bioactivity

IV.9.1.Evaluation of antioxidant activity

Reduction of the 1,1'-diphenyl-2-picrylhydrazyl (DPPH)

Radical is one of the most commonly employed methods and serves as the initial approach to assess the antioxidant activity of a molecule **(Shahidi and Zhong., 2015).**

Principe: The free radical scavenging method using DPPH is widely employed to assess the antioxidant activity present in plant extracts. Antioxidants function by reacting with the DPPH radical, initially indicated by its purple color, through hydrogen atom donation, resulting in its conversion to a yellow compound. The efficacy of antioxidants can be evaluated by measuring the decrease in DPPH absorption at a wavelength of 517 nm. This technic holds significant importance in studying and evaluating antioxidant activity **(Chebrou el *al*., 2022).**

Materials and reagents: Ethanol (Biochem Chemopharma), distilled water, L-ascorbic acid (Sigma-Aldrich), and 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich), spectrophotometr (SHIMADZU1800).

Procedure: In this assay, equal volumes of 1 mL of the sample and a 0.1 mM solution of DPPH were mixed and incubated at room temperature in darkness for 30 min. Absorbance was measured at a wavelength of 517 nm. Ascorbic acid was employed as the standard compound in this test, and the inhibition percentage was calculated using the following equation **(Gheraissa., 2022).**

Percentage Inhibition: (A0 – A1) / A0 × 100

A0: Absorbance of the control.

A1: Absorbance of a test or standard sample.

The concentration of extract/control for 50% inhibition (IC50) is determined by plotting the percentage inhibition against the concentration **(Tukappa et *al*., 2015).**

IV.9.2. Evaluation of antibacterial activity

Principe: This method involves placing a sterile disc soaked in polysaccharides extract into a bacterial lawn at the initial stages of its growth, allowing us to measure the zone where bacteria failed to proliferate. Based on this zone, we determine the inhibition diameter that

reflects the antibacterial activity of PSRR. The antibacterial activity of aqueous extracts of PSRR was assessed using the disc diffusion technic **(Ait EL hadj & Haddar., 2020).**

Materials and reagents: filter paper (Wattman's), Muller Hinton agar, petri dishes, Pasteur pipette, the Bunson burner nozzle, *Salmonella enteric* ATCC6017, *Escherichia coli* ATCC25922 , Amoxiciline (BIOCLAV,1g/125mg) , Gentamicin(GMN).

Procedure: The antibacterial effectiveness of the biofilms samples was assessed using the agar diffusion method, as described by (**Ojagh et *al*., 2010)**. The evaluation of the biofilms' antibacterial activity was carried out against 3 bacterial strains: *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 14028) . These strains were provided by the Pasteur Institute: Regional Veterinary Laboratory of Laghouat (RVLL). The strains were stored at a temperature of +4°C, with a renewal of these strains every 24 h on nutrient agar at 37°C.

The pathogenic strains were activated through incubation In a nutrient broth (Merck, Germany) at 37°C for 18 h. Following this, 100 µl of the bacterial suspension was evenly spread on nutrient agar (Merck, Germany), and the films, cut Into 1cm² squares, were placed on the agar surface. The petri dishes were then incubated at 37°C for 24 h. The results, Indicated by the diameter of the Inhibition zones, appeared as clear regions surrounding the biofilms (mm) **(Mahcene.Z., 2021)**.

IV.10. Application of biofilm-forming solutions as biopackaging

IV.10.1. Cheese coating

The impact of cheese coating using film-forming coating solutions was studied by immersing three cheese slices at each stage for 30 s. Subsequently, excess coating and coated cheese slices were dried at 20 °C for 30 min. The coated slices were stored in the refrigerator at a temperature of 5 ± 0.5 °C and a relative humidity of 75% for 17 days. Following this, the same steps were repeated, and the coated slices were stored at room temperature for 22 days.

IV.10.1.1 Physico-chemical characterization of coated cheese

A. PH

Principe: The pH scale measures the hydrogen ion (H+) activity in solutions and is utilized to determine their acidity, alkalinity, or neutrality. It serves as a fundamental indicator in chemistry and life sciences for assessing the properties of various solutions **(Mahcene. Z., 2021)**.

Materials and reagents: Gruyere cheese (superMarket), pH paper , distilled water.

Procedure: A study was conducted to assess the impact of biofilms on the acidity level of cheese during storage. PH strips were immersed in a cheese solution prepared by dissolving cheese pieces in distilled water. Subsequently, the color change of the strips was observed and compared to a set of known colors that change according to pH levels, in order to determine the extent of biofilm influence on the acidity level in cheese **.**

B. Weight loss

A study was conducted to evaluate the impact of bioactive coatings on cheese weight during storage. Initial and final weights were measured for groups of coated and uncoated cheeses by balance every 3 days over a period of 21 days. Bioactive film formation solutions were used as experimental groups, while the control group consisted of uncoated cheese **(AOAC., 2000)**.

C. Sensory analysis of coated cheeses

The coated cheeses underwent analysis after the storage period to assess their visual appearance, texture (visual structural integrity), as well as color and gloss evaluations **(Salhi & Marif., 2020).**

IV.10.1.2. Toxicological caracterization of coated cheese

The coated cheese samples undergo oxidation at a storage temperature of 4°C for 10 days. Oxidative changes can lead to unpleasant flavors, destruction of valuable nutrients, and the production of toxic compounds.

A. Peroxidation lipidic

Principe: The oxidation of lipids is catalyzed and accelerated through various mechanisms, including the production of singlet oxygen, the generation of free radicals and active oxygen, whether enzymatically or non-enzymatically. The assessment of lipid oxidation in cheese involves determining the peroxide value in cheese samples using the method recommended in Association of Official Analytical Chemistry (AOAC) standard.This method relies on treating cheese in a solution of acetic acid and chloroform along with a potassium iodide (KI) solution for the iodine titration, using a standardized solution of sodium thiosulfate (Na2S2O3) to titrate the released iodine **(Mahcene.Z., 2021).**

Materials and reagents: Gruyere cheese (superMarket),, acetic acid (CH3COOH), chloroform, potassium iodide solution, starch indicator, 0.01N sodium thiosulfate solution, 250 ml beaker, graduated burette, erlenmeyer flask, distilled water.

Procedure: This process commences by dissolving approximately 1g of cheese in a mixture comprising 5 mL of chloroform and 7.5 mL of acetic acid. The mixture is then left in darkness for 10 min, following which a 1% starch indicator solution is added to ascertain the endpoint. The liberated iodine quantity is determined, and subsequently standardized using a 0.02 M solution of sodium thiosulfate. Following this, the peroxide value (PV) is determined as outlined below :

$$\mathbf{P} = (\boldsymbol{V} - \boldsymbol{V0}) * \boldsymbol{N}/\boldsymbol{g}\ \textit{of lipids}$$

Where**V0** is the volume of sodium thiosulfate (ml) required to titrate the blank; **V** is the volume of sodium thiosulfate (ml) needed to titrate the sample;

N is the normality of the sodium thiosulfate solution.

B. Protein oxidation

Principe : The vital role of the sulfhydryl group (SH) and disulfide bonds (S-S) lies in supporting the structure and biological functions of native proteins. The impact of varying doses of edible films used to coat cheese on the oxidation process of sulfhydryl groups in proteins is determined using the Ellman method **(1959).**

Materials and reagents: Gruyere cheese (superMarket), tris-EDTA,DNTB, tubes ,centrifuges (6-15,N°121047) , spectrophotometr (SHIMADZU1800).

Procedure: The procedure commences by blending 0.2 mg of each sample with 4 ml of TrisEDTA solution, having a pH of 8, and adding 0.08 ml of 10 mM DNTB solution. This mixture is then activated for 20 min at room temperature. The control sample contains 0.03 mg of the original sample and the same volume of Tris EDTA solution. Subsequently, the mixture is centrifuged at 3000 rpm for 10 min, and the absorbance of the mixture is measured using a UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) at a wavelength of 412 nm. The concentration of the thiol group (CGS) is calculated using the following equation:

$$\mathbf{CGS} = (\mathbf{A} - \mathbf{B} - \mathbf{0.03})\ \mathbf{1.52mM}$$

Where A and B are the absorbance values of the sample and blank, respectively. **(Mahcene, Z., 2021)**:

CHAPTER V

Results and discussion

Conclusion

This study investigated the feasibility of manufacturing biodegradable food films using galactomannan extracted from the seeds of *Retama raetam*, a type of legume harvested in the El Oued province, Algeria. This sugar is water-soluble and serves as an alternative to galactomannan. Multiple experiments were conducted on *Retama raetam* seed extract (PSRR), including studies on total sugar concentrations, neutral sugars, proteins, and polyphenols, in addition to employing a developed technic.

Biodegradable biofilms were produced in the presence of the biopolymer and glycerol, and various tests were performed on them to measure thickness, opacity, moisture content, water contact angle, and water solubility, along with studies on biological activities such as antioxidant activity, antibacterial activity, and their application on cheese with a study of its physical properties. The results of this study showed that the biodegradable biofilms have good properties such as high solubility, appropriate thickness, and color, in addition to their strong activity against bacteria strains (E. Coli and Salmonella). The biofilm also exhibited significant antioxidant activity.The study proved that coating cheese with the biodegradable biofilm successfully preserves its safety, texture, color, and physical properties, and that the quality of the manufactured biodegradable biofilms is greatly affected by the structure of the raw material used. This is considered a cost-effective method for food preservation and can be used as a simple dipping layer suitable for local vendors.

Given the results obtained and the importance of the topic, this study is considered preliminary and can be used to improve the development protocol and enhance biodegradable biofilms. These identified properties of the membranes should be subject to further laboratory investigations, opening possibilities for the exploitation of these plants well-adapted to desert climates.

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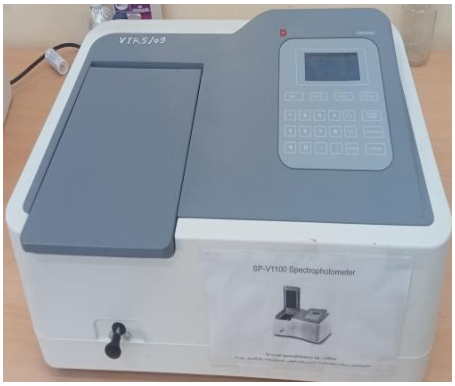
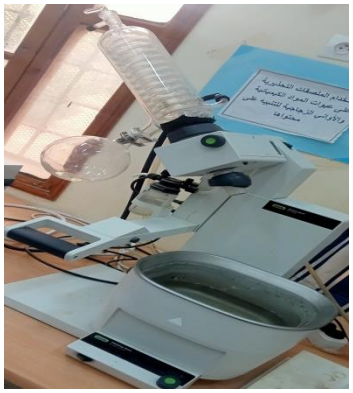
Annex

Annex 01

		
Desiccator with porcelain plate	**Spectrophotometer Visible**	**Refractometer**
		
Hotplate and stirrer	**Centrifuges Clinic**	**Conductivity meter**
		
Distillation Rotavaporator	**Heat bath**	**PH meter**

Annex 02

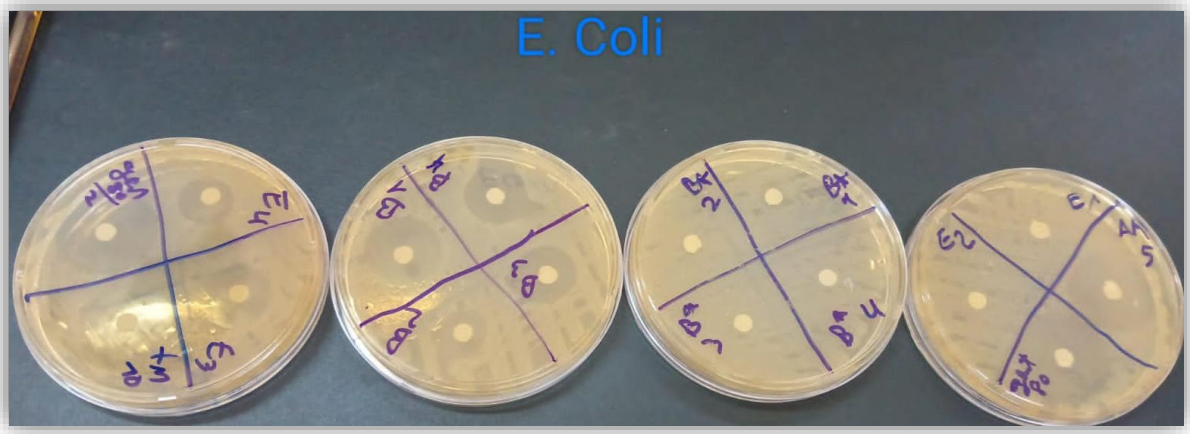


Figure 01: the diameters of the inhibition zones against *E. coli*.

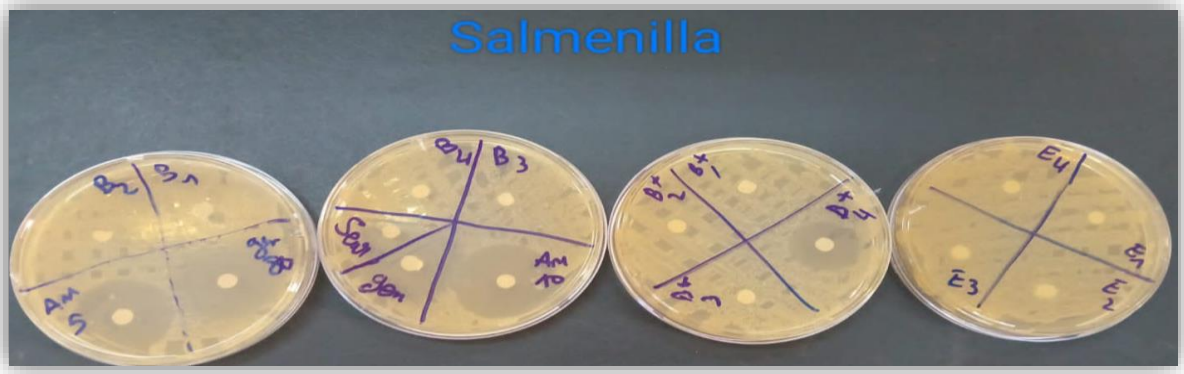


Figure 02: The diameters of the inhibition zones against *Salmonella enterica*.

Negative Control	Positive control	B1%	B1,5%

Cheese packaging with biofilm at 25°C.

Negative control	Positive control	B1%	B1,5%

Cheese packaging with biofilm at 4°C.

Cheese Gruyere.

Annex 03

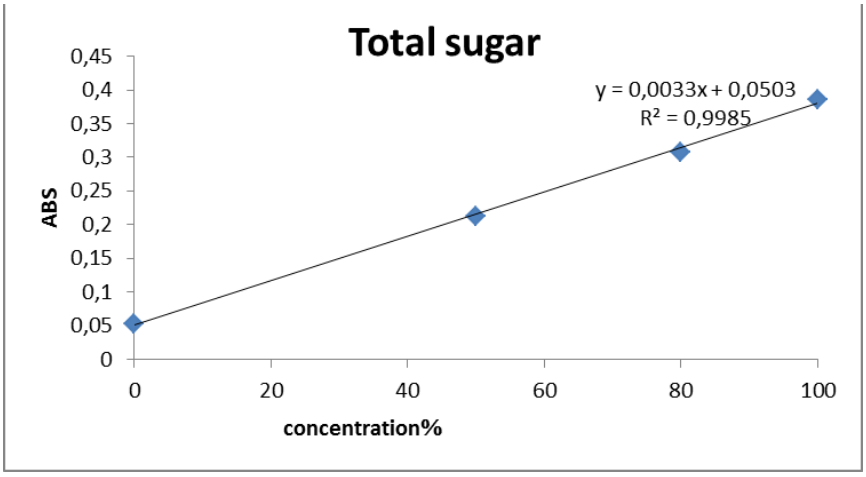


Figure 03: Calibration Curve for Total Sugars.

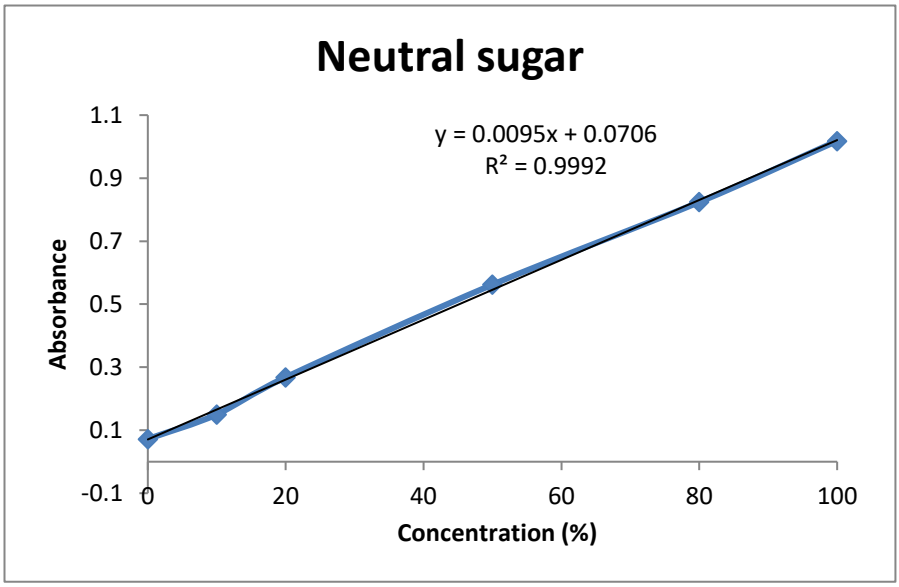


Figure 04: Calibration Curve for neutral sugar.

Annex 04

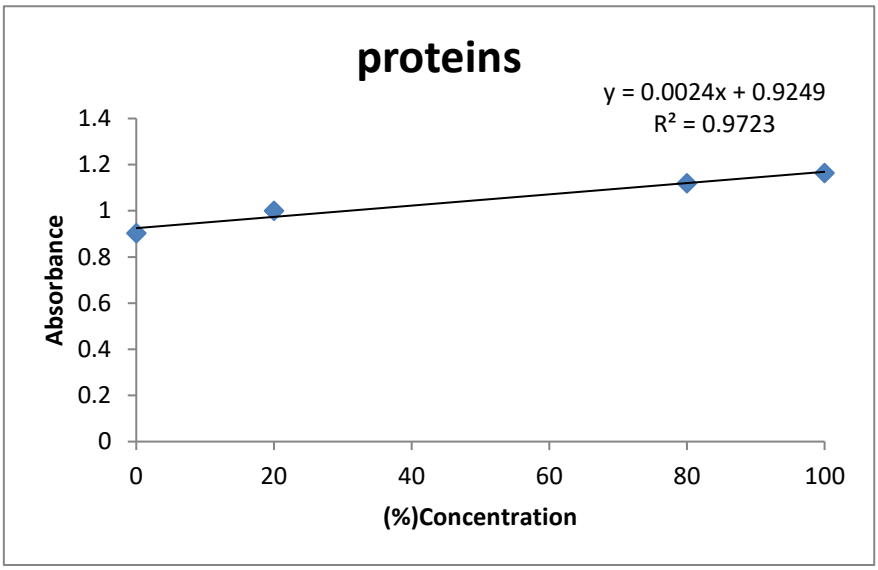


Figure 05: Calibration Curve for protein.

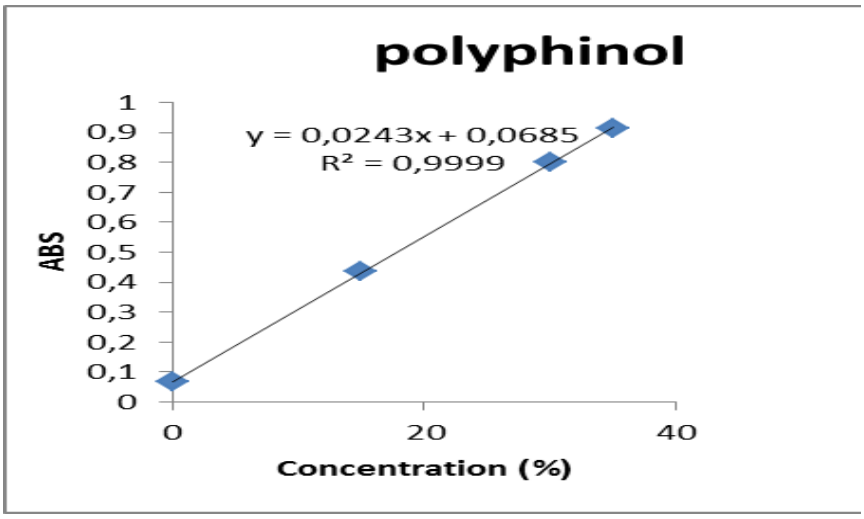


Figure 06: Calibration Curve for polyphenol.

Annex 05

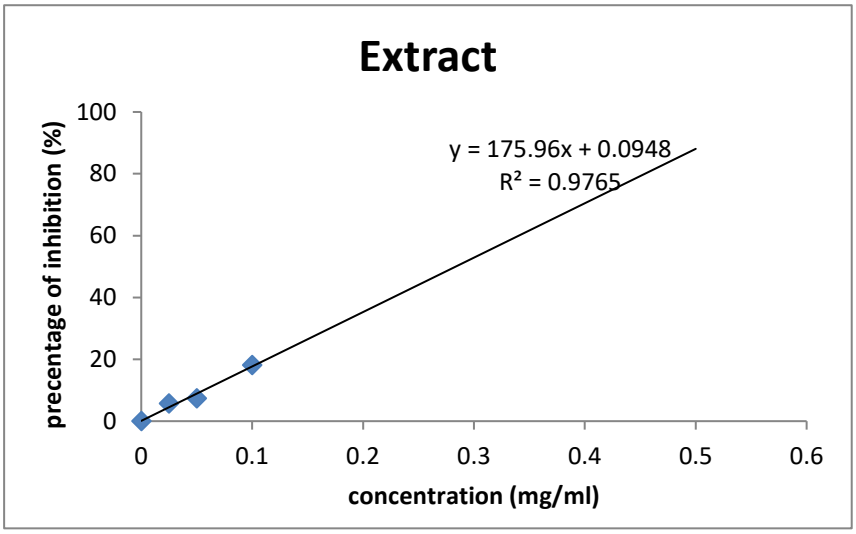


Figure 07: Inhibition percentage % as a function of PSRR extract concentration (mg /ml) .

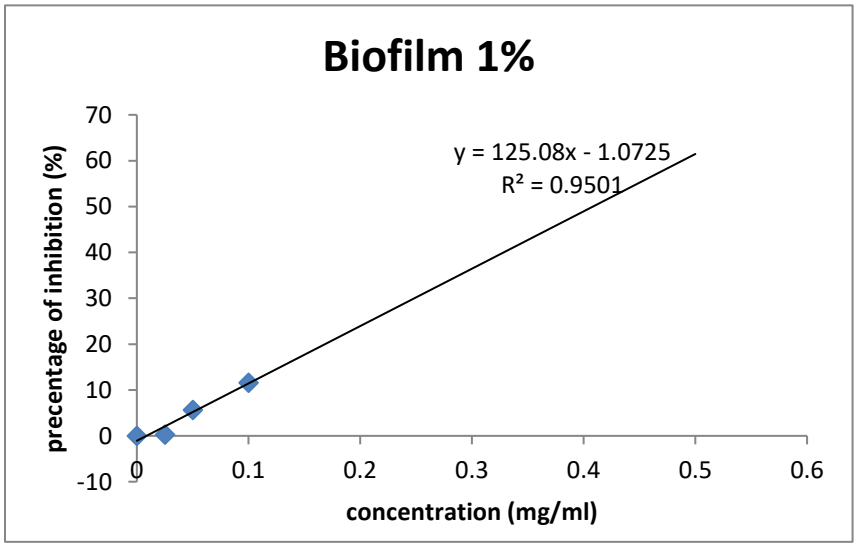


Figure 08: Inhibition percentage % as a function of biofilm 1% extract concentration (mg /ml).

Annex 06

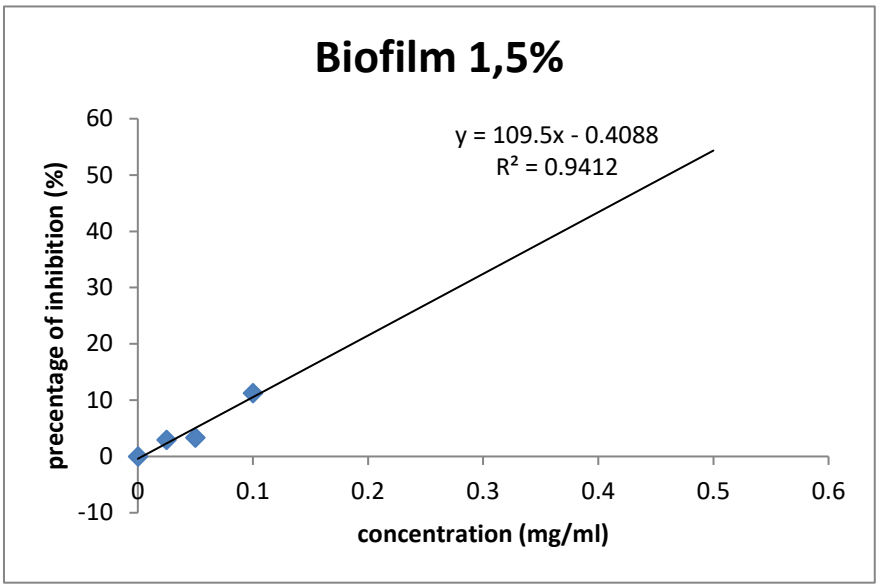


Figure 09: Inhibition percentage % as a function of biofilm 1,5% extract concentration (mg /ml).

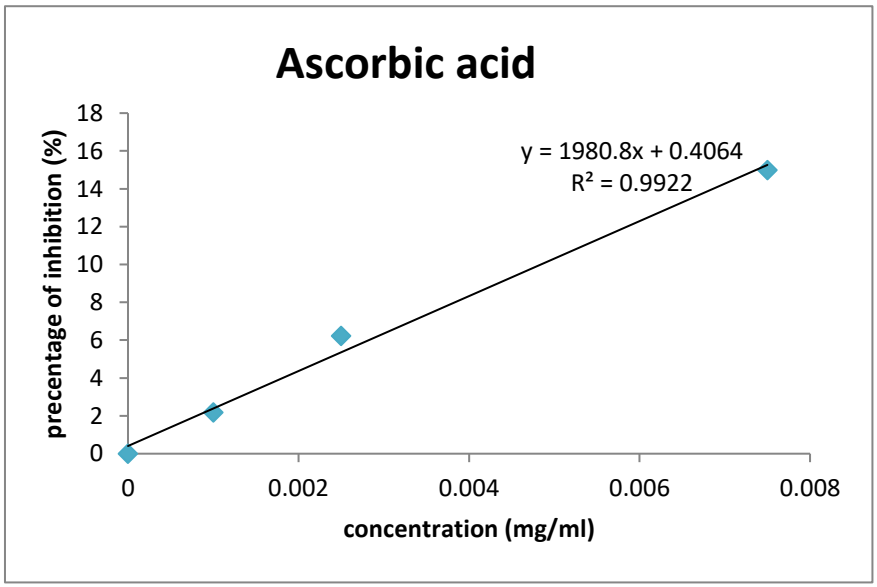


Figure 10: Calibration curve of ascorbic acid for the DPPH.