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END OF STUDY THESIS

In view of obtaining the Academic Master's Degree in Biological Sciences

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THEME

Exploitation of the brain-gut axis: Role of *Plantago albicans* polysaccharides in aluminium-induced neurotoxicity

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إلى نفسي بعد مسيرة دراسية دامت سنوات (2007,2025) حملت على طياتها الفرح ، التحديات ، التجارب و الدروس في الحياة و ها انا اقف الان على عتبة حلمي تخرجت بعد رحلة طويلة لم يكن فيها الأمر سهلا و لا الطريق كان محفوفا بتسهيلات لكنني فعلتها لأنه كان الثبات فيها اختياري كنت الداعم و الملهم لذاتي دائما و النور الذي مشى به قلبي وسط عتمة الأيام كل خطوة خطوتها و كل تعب ، ذرفته عيناي أحيانا في هذا المشوار كانو شاهدا على أن هذا نجاح صنع من روحي التي لم تعرف الاستسلام التي أمتن لقوتها التي كانت تنتشلني من لحظات العثرات و ظروف الصعبة و تمضي بي نحو النهوض من جديد و إيجاد حلول بشتى طرق بورك هذا الطريق الذي زرعته باليقين و التمسك و سقيته بالاجتهاد و الاصرار حد الوصول.

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إلى زميلاتي العزيزات في العمل: خولة(بيبا)، فاطمة، وأميرة كنتنّ الضوء الذي خفّف عني ثقل الأيام، والرفقة التي كانت مليئة بالدعم، بالضحك، وبالصدق... لن أنسى لحظاتكنّ الصافية ما حييت.

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موفق انصاف

Abstract

Exploitation of the brain-gut axis: Role of *Plantago albicans* polysaccharides in aluminium-induced neurotoxicity

Abstract

This study aims to investigate the gut-brain axis using polysaccharides of *Plantago albicans*, and to evaluate its effect on the gut microbiome and neurobehavioral responses in aluminum chloride induced Alzheimer's disease in animal model. Chemical analysis using colorimetric assays revealed that the extracted fraction, named PAP, has concentrations of 72.92% in total sugars, 64.53%, in neutral sugars, approximately 5% in protein, and a very low polyphenol content of 1.21%. FTIR results showed characteristic peaks indicating the presence of functional groups related to polysaccharides, such as out-of-plane vibrations of C–H and O–H at 600 and 650 cm^{-1}. *In vivo* experimentation was conducted on four groups of six Wistar rats. The study groups were named N, T, C-, and C+ indicating the individuals that received standard diet, PAP, $AlCl_3$, and PAP with $AlCl_3$, respectively. In vivo biological assessments on W. albino rats exposed to aluminum chloride indicated that *Plantago albicans* polysaccharides extract (PAP) partially improved oxidative balance in the brain, as evidenced by non-significant reduced MDA levels and a increase in GSH; $p > 0.05$. Conversely, a significant decrease in GSH was observed in some healthy rats following PAP administration ($p \leq 0.05$), suggesting a possible pro-oxidant effect in T group. The results showed that polysaccharides from *Plantago albicans* possesses antioxidant and anti-inflammatory properties, which positively influenced the rat's neurobehavioral performance and contributed to restoring gut microbiome balance. Moreover, colon sections indicated a reduction in submucosal thickness in C- group and an increase in mucosal thickness in C+ and T groups, indicating the positive effect of PAP on gut health. Additionally, the intestinal health index was positively correlated with higher antioxidant levels (GSH) in the kidneys and heart ($p \leq 0.05$), reflecting the relationship between the gut health and the oxidative stat of the individual. Microscopic examinations showed that the aluminum chloride group (C^-) suffered from thickening of blood vessel walls, surrounding hemorrhages, neuronal cell death, abnormal cell morphology, as well as infiltration of astrocytes and lymphocytes. In contrast, the C+ group exhibited a normal cerebral cortex and slight capillary proliferation, indicating the protective and therapeutic effect of PAP against aluminum-induced brain damage.

Keywords: *Plantago albicans*, polysaccharides, gut-brain axis, Alzheimer's disease, aluminum chloride.

استغلال محور الدماغ والأمعاء: دور عديدات السكاريد في*Plantago albicans* في السمية العصبية الناجمة عن الألومنيوم

الملخص

تهدف هذه الدراسة إلى استكشاف محور الأمعاء–الدماغ باستخدام متعدد السكريات المستخلص من *Plantago albicans*، وتقييم تأثيره على ميكروبيوم الأمعاء والاستجابات السلوكية العصبية في نموذج حيواني لمرض الزهايمر المُحفز بكلوريد الألمنيوم. أظهرت التحاليل الكيميائية باستخدام الطرق اللونية أن الجزء المستخلص، والذي أُطلق عليه اسم PAP، يحتوي على نسب تركيز بلغت 72.92% من السكريات الكلية، 64.53% من السكريات المحايدة، حوالي 5% من البروتين، ونسبة منخفضة جداً من الفينولات (1.21%). أظهرت نتائج تحليل FTIR وجود قمم مميزة تشير إلى وجود مجموعات وظيفية مرتبطة بمركبات متعدد السكريات، مثل اهتزازات C–H و O–H خارج المستوي عند 600 و650 سم$^{-1}$.تم إجراء التجارب الحية على أربع مجموعات من ستة فئران من نوع ويستر. تم تسمية المجموعات بـ N وT وC− وC+، للدلالة على الفئران التي تلقت النظام الغذائي القياسي، PAP، كلوريد الألمنيوم، وPAP مع كلوريد الألمنيوم، على التوالي. أظهرت التقييمات البيولوجية الحية على الفئران المعرضة لكلوريد الألمنيوم أن مستخلص متعدد السكريات من Plantago albicans (PAP) قد حسّن جزئياً التوازن التأكسدي في الدماغ، كما يتضح من الانخفاض غير المعنوي في مستويات MDA وزيادة GSH ($p > 0.05$). وعلى العكس من ذلك، لوحظ انخفاض معنوي في GSH لدى بعض الفئران السليمة بعد إعطاء PAP ($p \leq 0.05$)، مما يشير إلى احتمال وجود تأثير مؤكسد في مجموعة T.أظهرت النتائج أن متعدد السكريات المستخلص من Plantago albicans يتمتع بخصائص مضادة للأكسدة ومضادة للالتهابات، مما أثّر بشكل إيجابي على الأداء العصبي السلوكي للفئران وساهم في استعادة توازن ميكروبيوم الأمعاء. إضافة إلى ذلك، أظهرت مقاطع القولون انخفاضاً في سماكة الطبقة تحت المخاطية في مجموعة C−، وزيادة في سماكة الطبقة المخاطية في مجموعتي C+ وT، مما يدل على التأثير الإيجابي لـ PAP على صحة الأمعاء.كما أن مؤشر صحة الأمعاء كان مرتبطاً إيجابياً بمستويات مضادات الأكسدة (GSH) في الكليتين والقلب ($p \leq 0.05$)، مما يعكس العلاقة بين صحة الأمعاء والحالة التأكسدية للفرد. أظهرت الفحوصات المجهرية أن مجموعة كلوريد الألمنيوم (C^-) عانت من زيادة في سماكة جدران الأوعية الدموية، ونزيف محيط بها، وموت الخلايا العصبية، وتشوه في شكل الخلايا، بالإضافة إلى تسرب الخلايا النجمية والخلايا اللمفاوية. في المقابل، أظهرت مجموعة C+ قشرة دماغية طبيعية وتكاثر طفيف في الشعيرات الدموية، مما يشير إلى التأثير الوقائي والعلاجي لـ PAP ضد تلف الدماغ الناتج عن الألمنيوم.

الكلمات المفتاحية: *Plantago albicans* ، متعدد السكريات، محور الأمعاء–الدماغ، مرض الزهايمر، كلوريد الألمنيوم.

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List of Abbreviations

IACUC	Institutional Animal Care and Use Committee
CHROMagar	Chromogenic Agar
SH	Sulfhydryl group
Tris	Tris(hydroxymethyl)aminomethane
ANOVA	Analysis of Variance
XLSTAT	Statistical software
M ± SEM	Mean ± Standard Error of the Mean
2 nitro 5 mercapturic	Chemical compound part
alcl	Aluminum chloride
CLT	Classic Labyrinth Test
C−	Negative Control
T	Test Group
GN	Gram-Negative
GBA	Gut-Brain Axis
ATR	Attenuated Total Reflectance
MDA	Malondialdehyde
TBA	Thiobarbituric Acid
DTNB	5,5'-Dithiobis-(2-nitrobenzoic acid)
Na2CO3	Sodium Carbonate
nm	Nanometer
Broyat	Crushed powder
ROM	Range of motion
P1	Position 1
E. col	Escherichia coli
MPS	Multi-Position System or Medical Processing System
UV-Visible	Ultraviolet-Visible
PH	Potential of Hydrogen
NaCl	Sodium chloride
P<0.05	Probability less than 0.05
SEM	Standard Error of the Mean
DTNB	5,5'-dithio-bis-2-nitrobenzoic acid
H_2SO_4	Sulfuric acid
HSD	Honestly Significant Difference
C+	Positive Control
N	Normal Group
T1	Test1
GP	Gram-Positive
FTIR	Fourier Transform Infrared Spectroscopy
TBS	Tris-Buffered Saline
TBARS	Thiobarbituric Acid Reactive Substances
GSH	Glutathione
DO	Densité Optique
IR	Infrared
Ratio	Proportion
A	Absorbance
Tamb	Room temperature
TIM	Thickness of Intestinal Mucosa
WG	Weight Gain

STHS	Step-Through Movement Time (or related to entry behavior)
STHE	Step-Through Stay Time (or related to exit behavior)
STES	Step-Through Exit Time
STEN	Step-Through Entry Time
PAP	Plantago albicans Polysaccharides
GN Medium	Gram-Negative Medium
RSM	Response Surface Methodology
MDA	Malondialdehyde
GSH	Glutathione
CFU	Colony Forming Units
k. pneumoniae	Klebsiella pneumoniae
APG IV	Angiosperm Phylogeny Group IV
AD	Alzheimer's Disease
Aβ	Amyloid-beta
APP	:Amyloid Precursor Protein
Ach	Acetylcholine
GABA	Gamma-Aminobutyric Acid
IL-6	Interleukin-6
TNF-α	Tumor Necrosis Factor alpha
SCFAs	Short-Chain Fatty Acids
Lps	Lipopolysaccharides
HPA	Hypothalamic–Pituitary–Adrenal axis
ENS	Enteric Nervous System
CNS	Central Nervous System
GBA	Gut-Brain Axis
GM	Gut Microbiota
VN	Vagus Nerve
NFTs	Neurofibrillary Tangles
PHFs	Paired Helical Filaments
NLRP3	NOD, LRR and Pyrin Domain-Containing Protein 3
NSPs	Non-Starch Polysaccharides
RS	Resistant Starc
ECM	Extracellular Matrix
GAGs	Glycosaminoglycans
FTIR	Fourier-Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
HPLC	High-Performance Liquid Chromatography
FAD	Familial Alzheimer's Disease
SAD	Sporadic Alzheimer's Disease
BBB	Blood-Brain Barrier
LPS	Lipopolysaccharides
MW	Molecular Weight

Table of units	
g	gram
mg	milligra
mL	milliliter
µL	microliter
%	percent = parts per hundred
v/v	volume/volume = ratio of volume to volume
w/v	weight/volume = ratio of weight to volume
min	minute
h	hour
µm	micrometer
cm	centimeter
°C	degree Celsius = unit of temperature
rpm	revolutions per minute = number of full rotations per minute
nm	nanometer
µmol/L	micromole per liter = 10^{-6} mol per liter
mol/L	mole per liter = unit of concentration (molarity)
M	molar = shorthand for mol/L (1 M = 1 mol/L)
UFC/mL	Colony forming unite per mililiter
Kg	Kilogram
S	Seconds

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Chapter III: Results and Discussion

Introduction

Over the past two decades, the gut-brain axis has emerged as a key subject in biomedical research, due to its essential role in mediating the communication between the gastrointestinal system and the central nervous system. This bidirectional interaction is facilitated through neural, endocrine, and immune pathways and is significantly influenced by the gut microbiota, a complex ecosystem of trillions of microorganisms. Studies have shown that certain gut bacteria are capable of producing neurotransmitters such as serotonin, dopamine, and gamma-aminobutyric acid (GABA), thereby influencing mood, stress responses, and cognitive functions (Dinan & Cryan, 2017). Recently, several studies have highlighted a potential link between alterations in gut microbiota composition and the development of neurodegenerative diseases such as Alzheimer's disease. Specifically, Alzheimer's patients often show a significant reduction in microbial diversity and a shift toward pro-inflammatory microbial species (Vogt et al., 2017). These dysbiotic changes may promote systemic neuroinflammation, thereby accelerating neurodegenerative processes (Zhang et al., 2023). Thus, the gut-brain axis represents a promising therapeutic target in the context of neurodegenerative diseases (Dinan & Cryan, 2017; Vogt et al., 2017).

Moreover, plant-based interventions are gaining interest as a means to modulate the gut microbiota and, by extension, the gut-brain axis (Kang, 2017). Phytochemical analysis of Plantago extracts has revealed a rich content of phenolic compounds, flavonoids, and glycosides, which exhibit antioxidant and anti-inflammatory properties. These bioactive compounds may support the growth of beneficial gut bacteria while reducing inflammatory signaling, thus contributing to a healthier gut-brain interaction (Yang et al., 2020). Preliminary studies in animal models suggest that Plantago extracts may exert neuroprotective effects, possibly by modulating immune responses and reducing oxidative stress in neural tissue (Kang et al., 2017; Yang et al., 2020).

Recent studies have shown the substantial role of plant polysaccharides in the enhancement of gut and brain health and cognitive abilities (Wang et al., 2022). The immune-regulating properties of plant polysaccharides highlight their anti-inflammatory and antiviral capabilities, which are mediated through interactions with the gut microbiota (Ma et al., 2025). These complex carbohydrates, which are prevalent in various plant sources, function as prebiotics by nourishing gut bacteria and maintaining the balance of the intestinal microbiome. For example, polysaccharides isolated from *Gastrodia elata* stimulate the growth of probiotics, including Bifidobacterium, Collinsella, Prevotella, and Faecalibacterium, while inhibiting the abundance of pathogenic bacteria, such as Shigella, Dorea, Desulfovibrio, and Blautia (Zhou et al., 2025). These modifications or improvements in the intestinal microbiome, thanks to plant polysaccharides, have been linked to improved cognitive abilities and favorable mental health outcomes (Wang et al., 2022).

This work focuses on the study of the gut-brain axis through the intestinal microbiota, in the case of Alzheimer’s disease. Plantago albicans, a species long used in traditional medicine for its gastrointestinal and anti-inflammatory benefits, offers a compelling candidate for further investigation for improving the wellbeing of the nervous system through the modulation of gut microbiota.

The presented document is structured into three chapters. The first chapter presents a review about Plantago species, polysaccharides, gut-brain axis, and Alzheimer’s disease. The second chapter details the experimental methodology, including the extraction and partial characterization of *Plantago albicans* polysaccharides, and the study of their effects on Alzheimer's disease using Wistar rats. The third chapter presents the main results obtained, followed by their analysis and discussion. A general conclusion and future research perspectives finalizes the presented work.

Chapter I

Bibliographic review

In this chapter, it is presented an overview of *Plantago albicans* L. and the gut brain axis, using Alzheimer's disease as an illustrative example.

I.1. *Plantago albicans* L.

The Plantaginaceae family is a relatively recent addition to modern plant taxonomy, encompassing around 90 genera and more than 1900 species. Notable genera in the family include Plantago, Digitalis, and Antirrhinum, known for their medicinal, ornamental, and economic value (Pingxuan Xie et al., 2023). It is considered one of the most important plant families used in the medical field. This family has been known since ancient times for its traditional uses. Externally, Plantago species have been applied as bandages for wounds, insect bites, and burns (HELIS, 2005). Internally, they have been used for their anti-inflammatory (Hassan et al., 2015), laxative, and antidiabetic properties (Xiaolong Ji et al., 2019), among many other therapeutic applications.

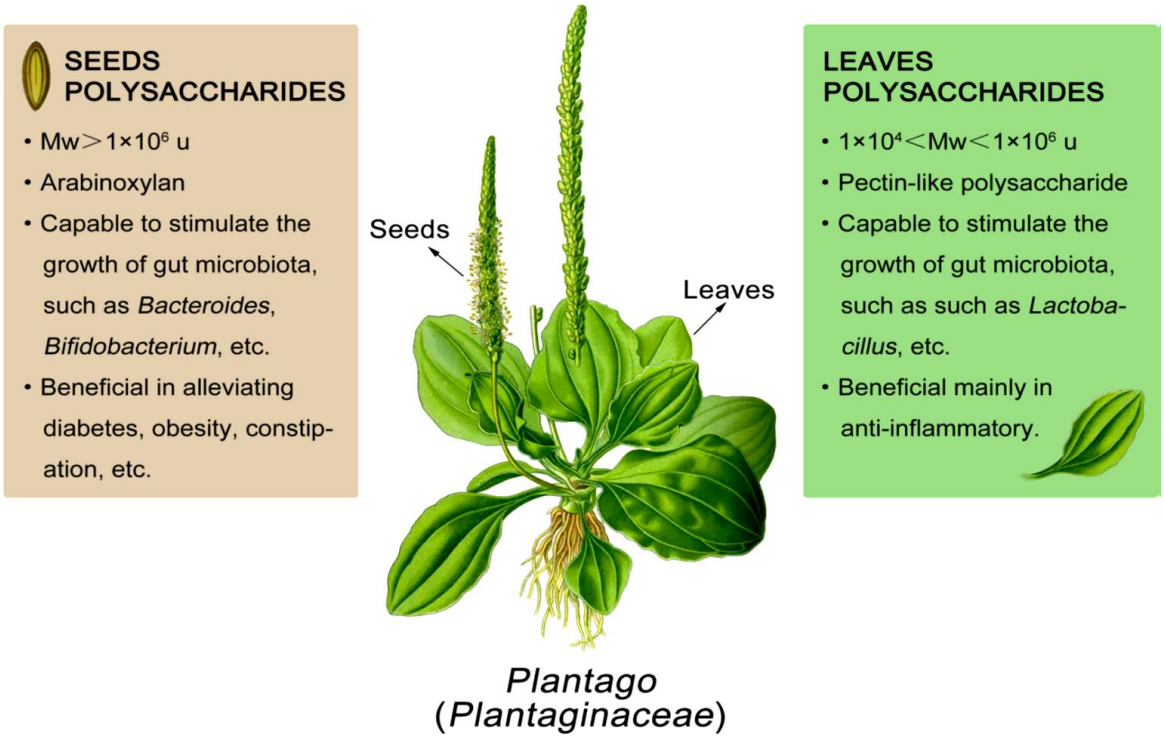

Figure1: Characteristics of leaves- and seed-derived polysaccharides from Plantago (Zhang et al., 2022)

I.1.1. Botanical description

Plantago albicans L. is a small annual plant, with a height ranging between 10 and 30 cm. It does not produce branched stems; instead, it grows as a compact tuft of leaves, resembling the growth pattern of onion plants. The leaves are lanceolate, elongated, and light green with a whitish tint due to a dense covering of white hairs. From the center of the plant arise spike-shaped

inflorescences, each borne on a long stalk that may exceed the height of the leaves. Each spike is composed of numerous very small flowers, each emerging from beneath a small green bract covered with fine hairs. The flowers are extremely small, not exceeding 2 mm, with a membranous corolla, and the ovary is fused at the base, forming a tubular structure. The stamens are carried on long filaments that extend beyond the corolla tube, and they bear large yellow anthers, which fall off shortly after the flower opens. (HELIS, 2005).

The systematic position of *Plantago albicans* L. according to Ozenda (1977), and Quezel and Santa (1962) is:

Kingdom	Plantae
Divisin	Angiospermae
Classe	Dicotyledonae
Order	Laminales
Family	Plantaginaceae
Genus	Plantago
Species	*Plantago albicans* L.

I.1.2. Traditional uses

In Algerian folk medicine, *Plantago albicans* is widely appreciated for its diverse therapeutic applications. The leaves are traditionally applied as topical bandages to promote wound healing and reduce skin inflammations. Moreover, leaf extracts are used to relieve joint pain and manage rheumatic conditions, indicating notable anti-inflammatory properties (Hemmami et al., 2023). The plant is also used to address urinary tract disorders (Nazim et al., 2024), digestive issues, particularly diarrhea (HELIS, 2005), and has a role in treating respiratory infections such as colds and bronchial inflammations (Ahmed Mohamed El-Bondkly et al. 2022).

I.1.3. Previous studies

Recent scientific studies have highlighted the multifaceted therapeutic potential of *Plantago albicans*, particularly in modern phytotherapy. Notably, its anti-obesity properties have been demonstrated by extracts of *Plantago albicans* that were effective in reducing weight gain and limiting fat accumulation in the liver and heart tissues of rats subjected to a high-fat diet suggesting a promising role in managing metabolic disorders (Samout et al., 2016). Furthermore, *Plantago albicans* has shown significant anti-inflammatory and analgesic effects justifying its traditional uses in relieving joint inflammations, rheumatic pain, and other inflammatory conditions (Hemmami et al., 2023). Another notable property is its litholytic activity, which refers to the ability to dissolve kidney stones, aligning with its traditional use in treating urinary tract disorders (Nazim et al.,

2024). Moreover, its antifungal activity is underlined, suggesting potential in combating fungal pathogens. Additionally, emerging evidence supports the plant's anticancer potential, thanks to its rich content of bioactive molecules that may inhibit the growth of certain cancer cells (Ahmed Mohamed El-Bondkly et al., 2022).

I.2. Polysaccharides

Polysaccharides are essential macromolecules which almost exist in all living forms and have important biological functions; they are getting more attention because they exhibit a wide range of biological and pharmacological activities, such as anti-tumor, immunomodulatory, antimicrobial, antioxidant, anticoagulant, antidiabetic, antiviral, and hypoglycaemic activities, making them one of the most promising candidates in biomedical and pharmaceutical fields. Polysaccharides can be obtained from many different renewable sources, such as plants, microorganisms, algae, and animals. Due to their physicochemical properties, they are susceptible to physical and chemical modifications leading to enhanced properties, which is the basic concept for their diverse applications in biomedical and pharmaceutical fields (Zhang et al., 2021).

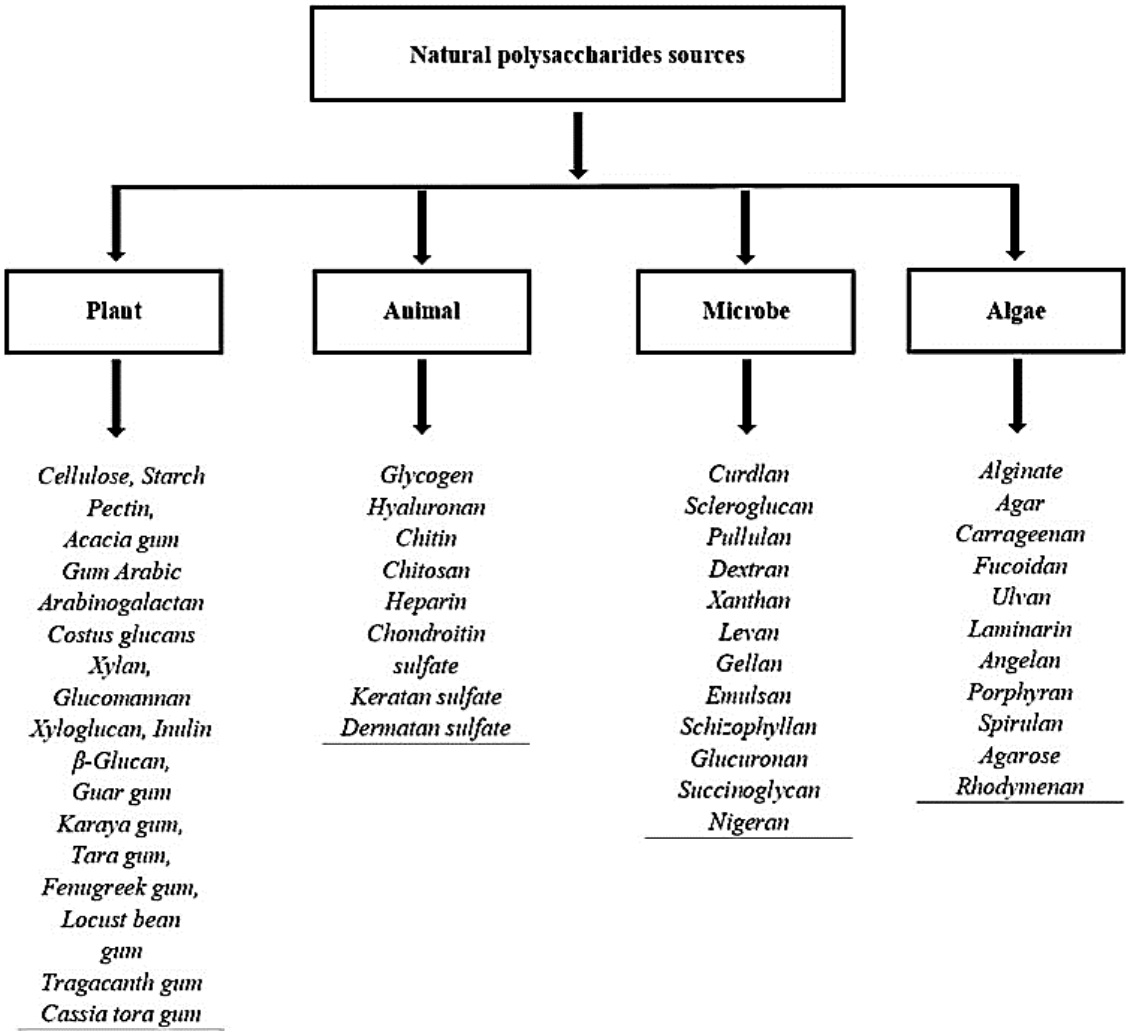

Figure 2: Examples of polysaccharides from different sources

Polysaccharides are the most abundant naturally occurring macromolecular polymers. Together with other biomolecules like proteins and nucleotides, polysaccharides are an essential component and exert many activities in the biological system, such as cell–cell communication, adhesion, and molecular recognition in the immune system. Polysaccharides, which belong to the third major class of biopolymers (carbohydrates), play crucial roles in many different physiological processes and tumor metastasis. They can also provide structure, protection, adhesion, and stimuli responsiveness. They also have crucial roles in the immune system, blood clotting, fertilization, pathogenesis prevention, and therapeutic efficacy (Zhang et al., 2021).

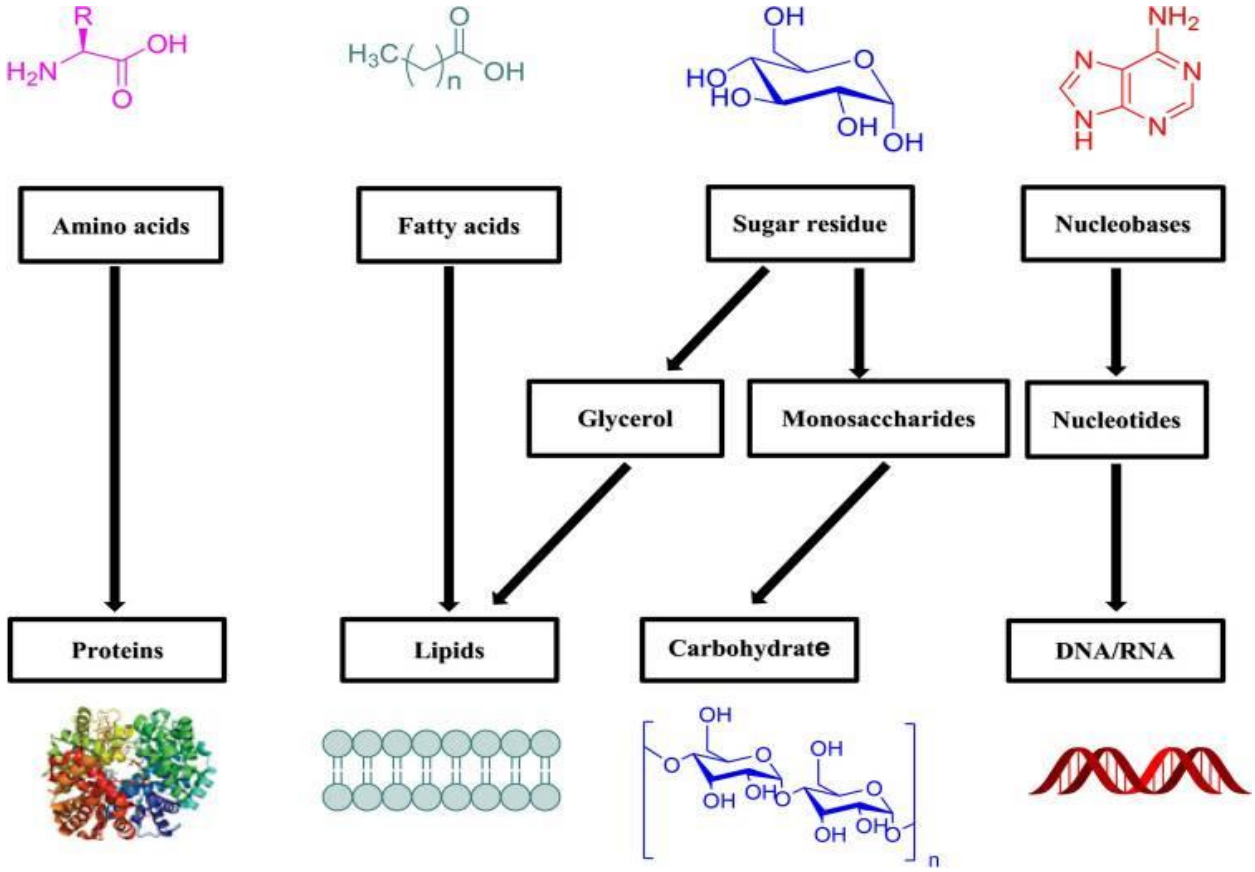

Figure 3: Major macromolecules founded in the body

I.2.1. Classification of polysaccharides

Polysaccharides, the most common form of carbohydrates that existed in nature, can be defined according to their chemical structure, which contains monosaccharide units that are linked by glycosidic bonds. They are either sugar residues that are glycosidically linked together or bonded covalently to other structures like peptides, amino acids, and lipids. Polysaccharides can be differentiated according to the nature of monosaccharide components, length of chains, and the branching of those chains. The glycosidic linkage through the anomeric carbon atom between the glycosidic bond donor and the acceptor forms either linear or branched chains, which makes them

distinguished from proteins and peptides since the latter have only linear chains (Abdel-Rahman et al., 2021).

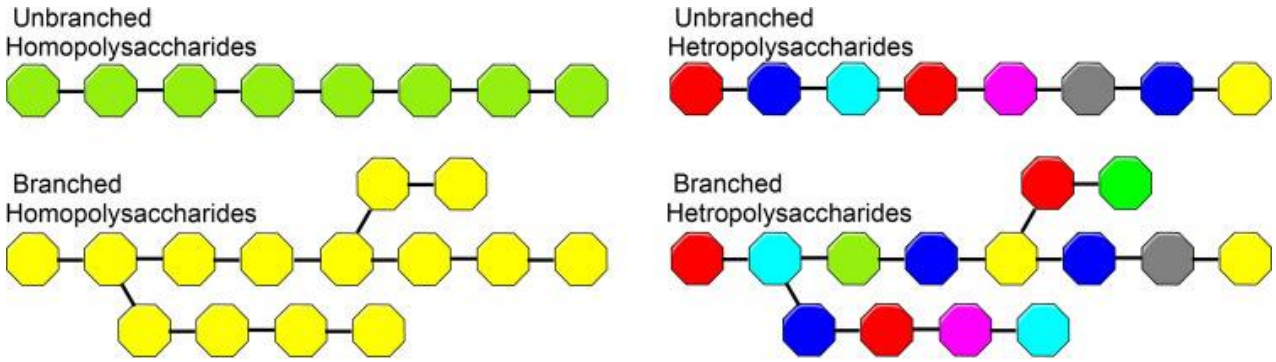

Figure 4: Branched and unbranched homopolysaccharides and heteropolysaccharides (Abdel-Rahman et al., 2021)

Homopolysaccharides are homoglycans that consist of the same monosaccharides, whereas heteropolysaccharides are heteroglycans that consist of different monosaccharides (Fig. 4). Glucans are glucose homopolysaccharides, while mannans are mannose homopolysaccharides. Hydrophilic groups like OH, COOH, and NH_2 groups can form a bioadhesive layer with epithelial and mucous membrane tissues. The most common constituent of polysaccharides is d-glucose; however, d-fructose, d-galactose, l-galactose, d-mannose, l-arabinose, and d-xylose are also frequent. Some monosaccharide derivatives found in polysaccharides include the amino sugars (d-glucosamine and d-galactosamine) as well as their derivatives (N-acetylneuraminic acid and N-acetylmuramic acid) and simple sugar acids (glucuronic and iduronic acids) (Abdel-Rahman et al., 2021; Zhao et al., 2020).

Polysaccharides naturally have storage properties like starch or structural properties like cellulose (Fig. 5), which provides physical structure and stability. Polysaccharides can also be classified based on polyelectrolyte to positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, and pectin). Another primary components of the cell surface and the cell-extracellular matrix are glycosaminoglycans (GAGs), namely heparin, heparan sulfate, hyaluronan, chondroitin sulfate, dermatan sulfate, and keratan sulfate; the most important GAGs polysaccharides in mammalian tissues (Abdel-Rahman et al., 2021).

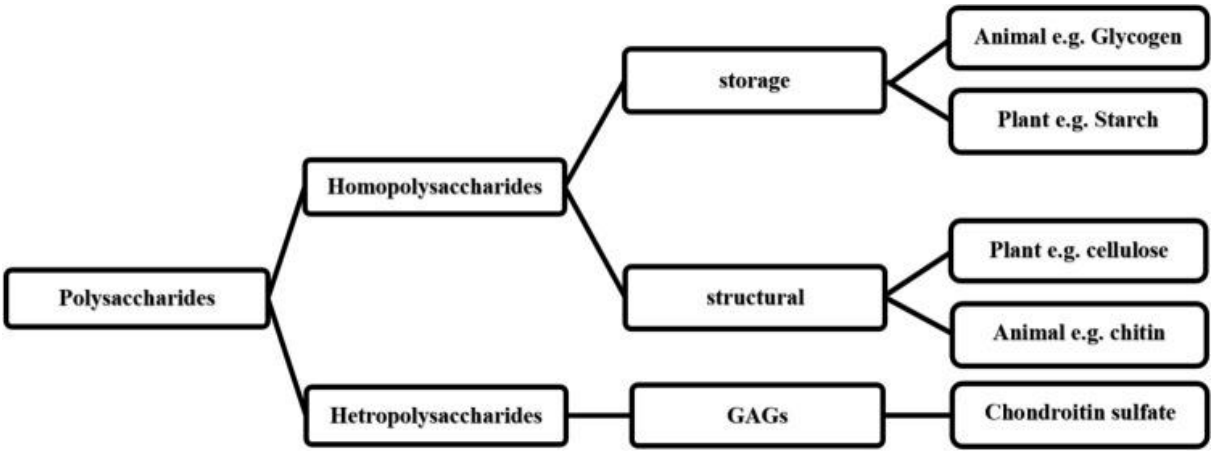

Figure 5: Classification of polysaccharides (Abdel-Rahman et al., 2021)

I.2.2. Physiochemical properties and applications

Polysaccharides exhibit a broad molecular weight range (10^3-10^7 Da), significantly impacting their rheological behavior and functional performance (Zhang et al., 2020). They can form either linear (e.g., cellulose) or highly branched (e.g., glycogen) architectures, influencing their solubility and interaction capabilities. The solubility of polysaccharides ranges from completely water-soluble (e.g., pullulan) to insoluble (e.g., chitin), depending on structural features like glycosidic linkages and degree of branching (Liu et al., 2021). In addition, polysaccharides many exhibit remarkable thermal stability (e.g., agar maintains gel structure up to 85°C), making them suitable for food processing and pharmaceutical applications (Nishinari et al., 2022). Furthermore, hydrophilicity and gelation capacity of polysaccharides is due to its possession of numerous hydroxyl groups enabling exceptional water-binding capacity (up to 100× their weight), thus facilitating hydrogel formation for wound dressings and drug delivery (Dai et al., 2023). Polysaccharides can form complexes with proteins, polyphenols, and drugs through hydrogen bonding and electrostatic interactions, enabling controlled release systems (Li et al., 2023).

The stability, hydrophilicity, and biodegradability, along with other properties like the diversity of the physicochemical properties of natural polysaccharides, provide the basis for its wide range of biological activities. It has been established that the biochemical and physical properties of polysaccharides have contributed to their biomedical applications (Loelovich, 2021). Their unique physicochemical properties make them indispensable in numerous biomedical and industrial applications. In tissue engineering, alginate/chitosan scaffolds show excellent biocompatibility for cartilage regeneration (Chen et al., 2022). Moreover, nanocellulose composites exhibit superior barrier properties while being fully biodegradable for sustainable packaging (Dai et al., 2023).

I.2.3. Polysaccharides and intestinal microbiota

Polysaccharides that contain many sugar monomers include starch and non-starch polysaccharides (NSPs) together with resistant starch (RS). Dietary polysaccharides are well known to have a wide range of biological benefits for bowel health. Gut microbiota and their fermentative products, short-chain fatty acids (SCFA), which have recently been highlighted as metabolic regulators, are thought to mediate the function of dietary complex carbohydrates and bowel health. Physiological impacts of non-starch polysaccharides (NSPs) and resistant starch (RS), both of which pass through the small intestine nearly intact and can be fermented by gut microbiota in the large intestine, are similar to each other. They exert a wide range of beneficial effects, including anti-inflammation, gut epithelial barrier protection, and immune modulation through both microbiota-dependent and -independent mechanisms. Bacterial polysaccharides usually found in the cell wall generally act as immune modulators, and host-derived polysaccharides not only protect host cells from pathogenic microbial neighbors but also affect overall intestinal health via interactions with gut microbes (Rajkumar, 2020).

Natural polysaccharides play an important role in alleviating intestinal inflammation, especially in inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. These compounds promote intestinal health through several biological mechanisms. First, polysaccharides stimulate the growth of beneficial bacteria in the gut, enhancing the balance of the intestinal microbiome. This balance contributes to strengthening the intestinal mucosal barrier, reducing intestinal permeability and preventing the leakage of harmful substances into the bloodstream. Second, polysaccharides exhibit anti-inflammatory effects by reducing the secretion of inflammatory mediators such as IL-6 and TNF-a and regulating the immune response in the gut. In addition, polysaccharides enhance the production of short-chain fatty acids (SCFAs) such as butyrate, which play an important role in strengthening the intestinal barrier and reducing inflammation (O'Connor et al., 2021).

Some plant polysaccharides, such as beta-glucan and pectin, also demonstrate the ability to restore immune balance and reduce the infiltration of immune cells into the intestinal wall (O'Connor et al., 2021). These polysaccharides play crucial functional roles in the body. First, they exhibit anti-inflammatory properties. In vitro studies have demonstrated that polysaccharides isolated from Plantago can reduce the levels of inflammatory mediators such as TNF-α and IL-1β, which are directly involved in the mechanisms of intestinal inflammation. Second, polysaccharides act as non-digestible dietary fibres that reach the colon, where they are fermented by beneficial gut microbiota (e.g., Bifidobacteria, Lactobacillus), leading to the production of short-chain fatty acids

(SCFAs) like butyrate. These SCFAs play a key role in strengthening the intestinal mucosal barrier and reducing inflammation (Zhao et al., 2014).

I.3. Review of the gut–brain axis

The GBA is a bidirectional communication system that connects the central nervous system (CNS) with the gastrointestinal tract (GIT). This communication involves neural pathways (such as the vagus nerve) (Iannone et al., 2019), as well as hormonal and immune mechanisms, in addition to the essential role played by the gut microbiota (the beneficial microorganisms residing in the gut) (Tarawneh & Penhos, 2023). This axis plays a key role in regulating digestive functions and immune responses and also influences mental health and cognitive processes, including mood, stress, and memory. Recent research indicates that dysregulation of this axis may contribute to the development of various neurological and psychiatric disorders, such as depression, anxiety, and Alzheimer's disease (AD) (Ashique et al., 2024).

I.3.1. Physiology of the gut–brain axis

The gut–brain axis represents an integrated physiological system that connects the central nervous system (CNS) with the gastrointestinal tract (GIT), playing a dual regulatory role in controlling the functions of both the brain and the gut. This axis relies on the complex interplay between several key components (Inan et al., 2025; Loh et al., 2024; Varesi et al., 2022):

- **Enteric nervous system (ENS):** Often referred to as the "second brain", the ENS is a mesh-like system of 200 to 600 million neurons embedded in the gastrointestinal system’s lining, which facilitates communication between the brain and the GM. It’s capable of autonomously regulating intestinal motility and secretions without direct input from the brain (Rusch et al., 2024).
- **Vagus nerve**(VN)**:** it is the tenth cranial nerve that extends from its origin in the brainstem down to the visceral organs, acts as a central communication pathway, transmitting sensory and motor signals between the gut and the brain, and plays a crucial role in gut–brain interaction (Rusch et al., 2024).
- **Gut microbiota:** The complex, nonpathogenic, active, diverse community of bacteria, fungi, archaea, parasites, and viruses that establish symbiotic relationships with the host constitutes the gut microbiome. Its participation in the production of neurotransmitters such as serotonin and GABA influences mood and immune responses and exerts both direct and indirect effects on the CNS (Dandamudi et al., 2024).

- **Hormonal and immune systems;** Hormones (e.g., cortisol) and cytokines contribute to the regulation of stress and inflammation, both of which have significant impacts on brain function and cognition (Rusch et al., 2024).

Due to the physiological integration, the GBA contributes to the maintenance of homeostasis and mediates adaptive responses to environmental and psychological stimulation. Any dysfunction in the GBA may contribute to the development of psychiatric disorders (such as anxiety and depression) or neurological diseases (such as AD and Parkinson's disease) (Rusch et al., 2024).

I.3.2. Pathways for GBA interactions

I.3.2.1. Neuronal pathways for GBA interactions

Recent literature highlights the presence of two major neuroanatomical pathways through which the gut communicates with the brain. The first pathway is the direct communication via the VN and the autonomic nervous system (ANS) located within the spinal cord. These structures transmit neural signals between the gastrointestinal tract and the brain in a bidirectional way. The second pathway is the ENS, often referred to as the "second brain", which independently regulates gastrointestinal functions. In coordination with the ANS and VN, the ENS facilitates mutual interaction between the gut and the brain (Shaik et al., 2020). Several studies have demonstrated that the gut microbiota can establish direct neural communication with the brain through VN stimulation and activation of afferent ENS neurons (Angelucci et al., 2019). Vagal activation exerts significant anti-inflammatory effects and positively influences microbiota balance, including the promotion of probiotic strains (Caradonna et al. 2024).

I.3.2.2. Immune pathway of GBA

The immune pathway is one of the most important paths through which the GM communicates with the brain. It plays a vital role in modulating immune responses and brain function. Immune cells in the gut produce cytokines. These cytokines can enter the bloodstream and reach the brain, where they influence neuronal activity and contribute to changes in behavior and mood. Moreover, the gut microbiota shapes the development and maturation of the immune system. Disruptions in microbial balance (dysbiosis) can lead to abnormal immune responses, which may negatively impact brain function (Ullah et al., 2023). In addition, the intestinal epithelial barrier prevents the translocation of microbes and toxins into the bloodstream. When this barrier is compromised, pro-inflammatory molecules can enter the circulation and reach the brain, potentially triggering neuroinflammation **(**Houser & Tansey, 2016).

I.3.2.3. Microbial pathway

Certain gut bacteria produce neurotransmitters such as serotonin (90% of body serotonin), dopamine, GABA, and norepinephrine, which influence mood and neurological functions (Rusch et al., 2024). Likewise, gut bacteria produce short-chain fatty acids (SCFAs) like butyrate, which help protect the blood-brain barrier and regulate neuroinflammation (Ortlek et al., 2024).

I.3.2.4. Hypothalamic–pituitary–adrenal axis pathway

The hypothalamic–pituitary–adrenal (HPA) axis is a fundamental part of the body's stress response and interacts with the gut microbiome in multiple ways. Dysbiotic gut microbiota can influence the HPA axis activity by reducing the production of metabolites such as SCFAs and tryptophan..., which are essential for neuroimmune signaling and stress regulation. In addition, cortisol, produced upon HPA axis activation, affects gut microbiota composition, potentially leading to dysbiosis. Activation of the HPA axis during stress increases glucocorticoids such as cortisol (Bertollo et al., 2025), which can disrupt gut microbiota composition and compromise intestinal barrier integrity, allowing microbial components like LPS to enter circulation (Lu et al., 2024).

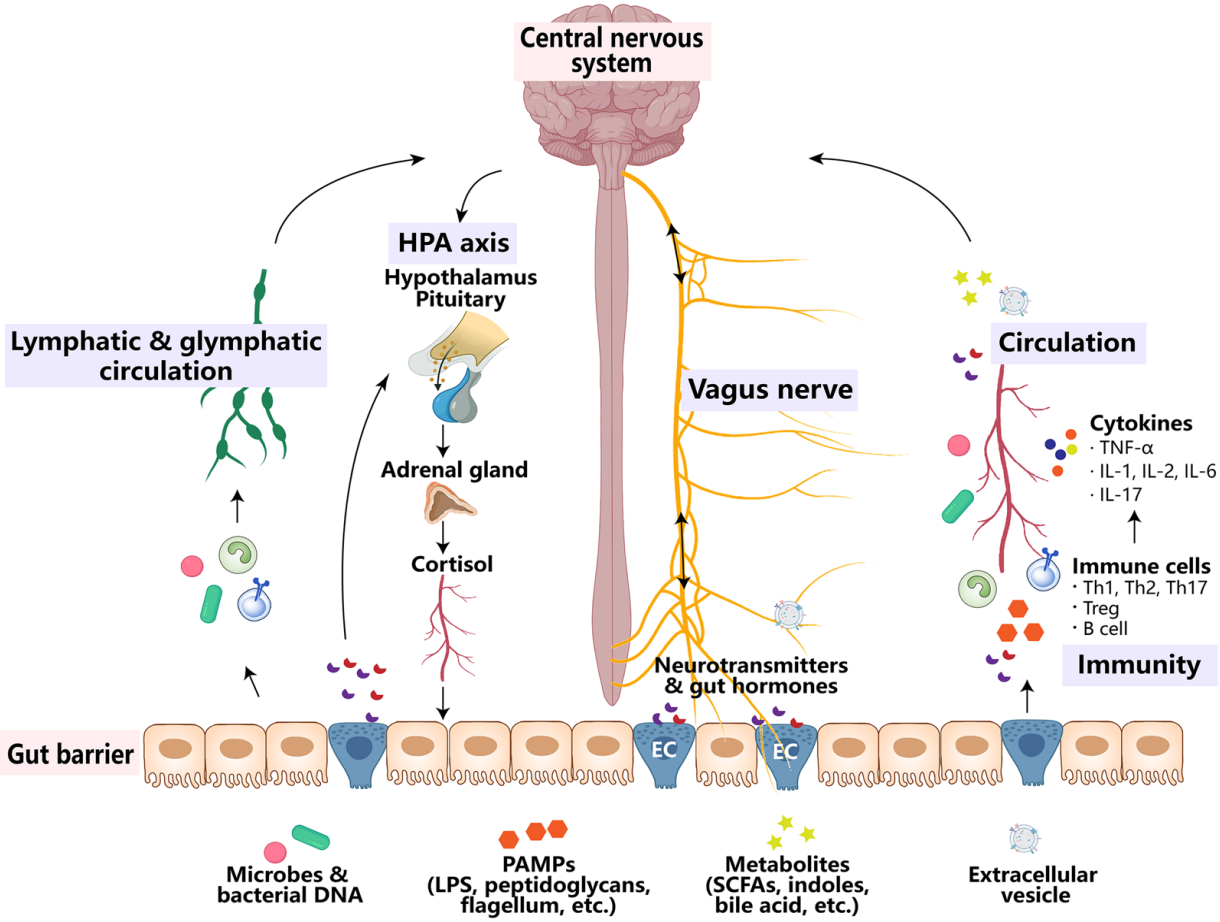

Figure 6: Overview of the microbiota-gut-brain axis (Ma et al., 2024)

I.3.3. Impact of GBA balance

The GBA plays a crucial role in regulating the immune system, where the balance of the GM affects neuroinflammation and the functions of glial cells (microglia) in the brain. Studies suggest that changes in the dysbiosis may contribute to the development of neurodegenerative diseases such as AD and Parkinson's (Loh and al., 2024). The gut microbiome also contributes to the production of important neurotransmitters such as serotonin, dopamine, and gamma-aminobutyric acid (GABA), as well as their precursors, tryptophan, which play a role in regulating mood, mental function, and cognitive function. An imbalance of these neurotransmitters is believed to be linked to disorders such as depression, anxiety, and hyperactivity (Inan et al., 2025). In addition, The gut microbiome affects the development of the central nervous system, including the maturation of neurons and the formation of the blood-brain barrier (Schächtle & Rosshart, 2021). Moreover, the GBA interacts with the HPA , affecting the body's response to stress. A dysbiosis can lead to increased cortisol secretion, influencing mood and behavior (Lu et al., 2024).

I.3.4. Neuroactive role of gut microbiome

The gut microbiome plays a crucial role in influencing brain function through various neuroactive mechanisms. Specific bacterial strains, including Lactobacillus and Bifidobacterium, are known to synthesize neurotransmitters such as GABA, dopamine, and serotonin, over 90% of which is generated in the gut. This production has a direct impact on emotional regulation and the body's response to stress, highlighting the importance of gut health in overall mental well-being (Clapp et al., 2017). The microbiome plays a significant role in the modulation of the HPA, which is responsible for regulating cortisol secretion in response to stress. On the other hand, an imbalance in microbial populations may lead to abnormal activation of the HPA axis, thereby contributing to the development of mood disorders such as anxiety and depression. Furthermore, gut microbiota generate short-chain fatty acids (SCFAs), such as butyrate, which are instrumental in preserving the integrity of the blood-brain barrier and mitigating neuroinflammation (Bertollo et al., 2025). Thus, both animal and human studies confirm that altering gut flora through probiotics improves behavior, mood, and cognitive function (O'Riordan et al., 2025).

I.3.5. Dysbiosis and Its Impact on Neuroinflammation

Dysbiosis, defined as the imbalance within the gut microbiome, is recognised as a significant factor influencing the onset of neuroinflammation. This condition induces alterations in the composition of gut bacteria, which in turn affects brain health through various mechanisms

(Zhao et al., 2023). Dysbiosis compromises the integrity of the intestinal barrier, permitting inflammatory molecules, such as lipopolysaccharides (LPS), to enter the bloodstream, thus increasing intestinal permeability (Leaky Gut). These molecules can subsequently traverse the blood-brain barrier, thereby instigating neuroinflammation. Inflammatory molecules engage with microglia within the brain, resulting in the release of cytokines, including IL-6 and TNF-α. These cytokines play a pivotal role in the promotion of neuroinflammation and neuronal damage, contributing to depressive symptoms and cognitive impairment (Bertollo et al., 2025; Bostanciklio, 2019).

Dysbiosis disrupts the synthesis of neurotransmitters such as serotonin and GABA, thereby influencing mood and behavior. This disruption may contribute to the emergence of psychiatric disorders, including depression and anxiety (Leigh et al., 2023). In addition, dysbiosis promotes the conversion of tryptophan into inflammatory pathways, notably kynurenine, which is associated with neuroinflammation and the progression of neurological diseases (Wiatrak et al., 2023).

I.1.4. Pathophysiology of Alzheimer's disease

As reported by the World Health Organisation (WHO), Alzheimer’s disease is a progressive neurodegenerative disorder that affects the brain, leading to a gradual decline in memory, cognitive function, and behavior. It is the most common cause of dementia in older adults. The disease often starts with mild memory loss and progressively worsens, eventually impairing communication, reasoning, and the ability to perform daily tasks. AD can be categorised into two main types familial (accounting for 1-5% of AD cases) and sporadic forms (over 95% of cases). Familial AD (FAD) is predominantly characterized by autosomal dominant genetic mutations in amyloid precursor protein (APP). Sporadic AD (SAD), also known as late-onset AD, usually manifests after the age of 65 and is influenced by a combination of genetic risks, environmental factors, and various comorbidities (Zhang and al., 2024).

The pathophysiology underlying mechanisms of Alzheimer's disease are not yet fully defined or clearly understood. However, several hypotheses have been proposed to explain the onset or progression of the AD, which are summarized as follows:

- **The cholinergic hypothesis**

This hypothesis suggests that AD results from the degeneration of cholinergic neurons in the basal forebrain. This leads to decreased levels of the neurotransmitter acetylcholine (ACh), resulting in memory impairment and other cognitive decline. Even after the use of cholinesterase

inhibitors during treatment, no reduction in Alzheimer's symptoms has been observed. This demonstrates that this hypothesis is merely part of the pathophysiology of AD's (Liu et al., 2024).

- **The Amyloid Hypothesis**

This theory is one of the most widely accepted theories explaining the mechanism of Alzheimer's disease. This hypothesis states that the primary cause of Alzheimer's is an imbalance between clearance and production of beta-amyloid (Aβ) 40 and 42, resulting from the cleavage of beta-amyloid by β- and γ-secretases. This leads to its accumulation in the blood, which becomes permeable to the brain, forming Aβ plaques. However, soluble Aβ oligomers are believed to be more toxic, causing neuronal membrane disruption, neuroinflammation, calcium imbalance, mitochondrial dysfunction, oxidative stress, and synaptic damage. Studies have also shown that plaque accumulation is a consequence, not a cause, of AD's disease, which underscores the need to investigate other causes and hypotheses to explore tau pathologies and other mechanisms (Al-Kuraishy et al., 2025).

- **The Tau Hypothesis**

In its normal state, tau is a transport protein between and across neurons, where it is responsible for phosphorylation and dephosphorylation. It is a fibre-associated protein regulated by kinases and phosphatases. In the pathological state, hyperphosphorylation of tau causes its detachment from fibers and microtubules, resulting in intracellular accumulation in the form of paired helical filaments (PHFs) and neurofibrillary tangles (NFTs). This abnormal accumulation leads to brain atrophy, impaired glucose metabolism, and typical and atypical symptoms of Alzheimer's disease (Chen & Yu, 2025).

- **The Neuroinflammation Hypothesis**

This hypothesis explains that the main cause of Alzheimer's disease is neuroinflammation due to the stimulation of immune microglial cells in response to the accumulation of beta-amyloid. Initially, they become less efficient over time and continue to release pro-inflammatory cytokines that damage neurons. Interactions between microglia and tau promote tau phosphorylation and spread between neurons, exacerbating neurodegeneration (O'Riordan et al., 2025).

- **The Microbiota–Gut–Brain Axis Hypothesis**

This hypothesis suggests the existence of an axis of communication between the brain and the intestine, where the latter is based on neuro-hormonal-metabolic connections. This axis is

affected by all external influences, such as psychological ones like fatigue, anxiety and stress, as well as diet. When this imbalance occurs, it causes a bacterial imbalance, which in turn weakens the intestinal wall and its permeability, allowing inflammatory mediators to cross the blood-brain barrier. As a result, glial cells are activated, which increases the level of neuroinflammation, cell damage and degeneration (Chandra et al., 2024).

- **The Metal Ion Hypothesis**

This hypothesis suggests that any imbalance in the levels of minerals, such as aluminium, in the blood results in an imbalance in the brain, allowing them to accumulate in amyloid plaques. This leads to memory loss due to the accumulation of mineral levels, cell atrophy and degeneration, as well as impaired cognitive function due to dysfunction of the synapses, especially the decrease in zinc and magnesium levels (Zhang et al., 2024).

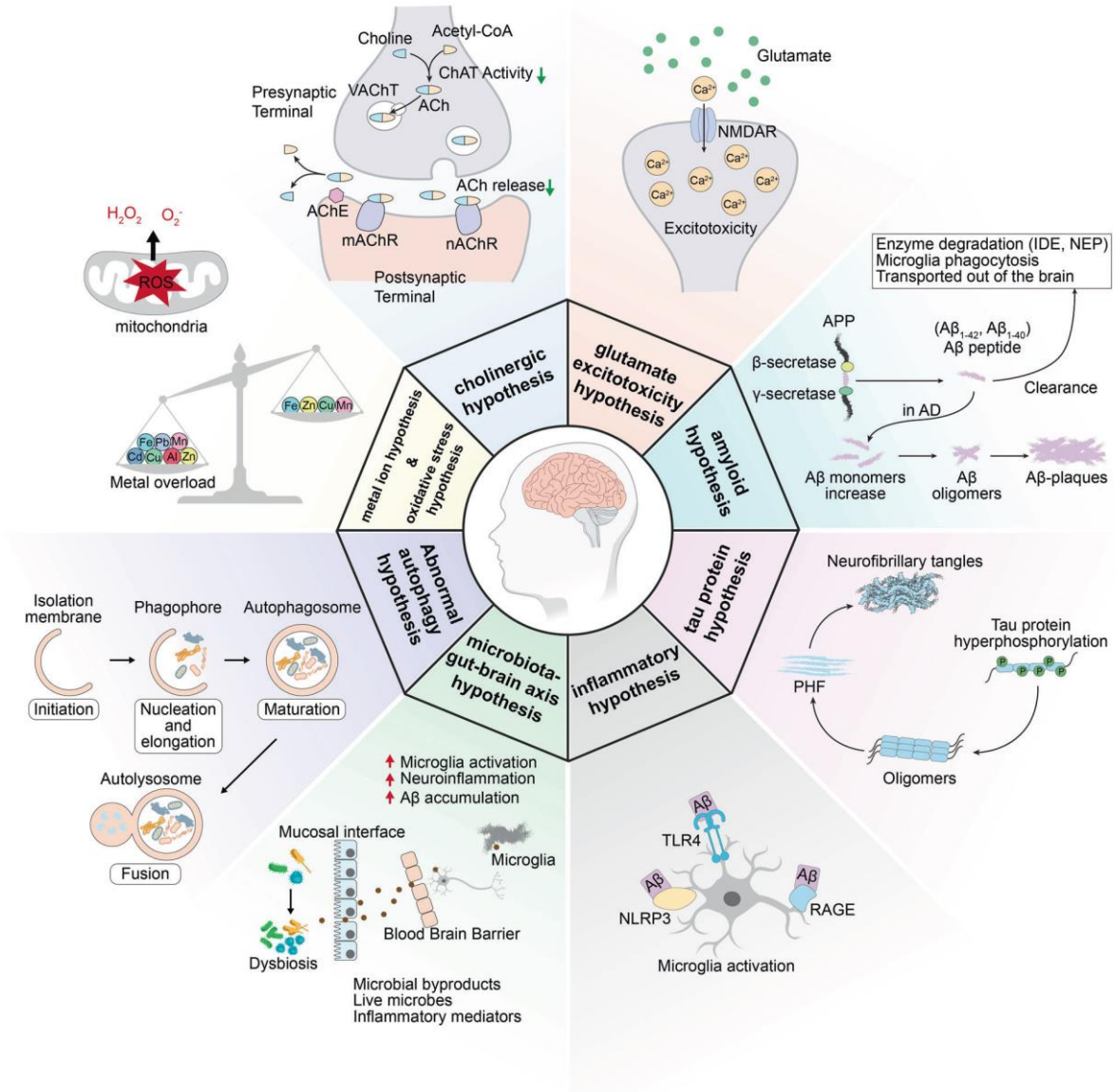

Figure 7: Diagram for the pathogenesis hypotheses of AD (Zhang et al., 2024)

Chapter II

Material and methods

This chapter deals with the principle of the study, the materials used, the study of water-soluble polysaccharides, and the evaluation of biological activities *in vivo*.

II.1. Principle of the study

The bidirectional relationship between the gut and the brain commonly referred to in many studies as the GBA, plays a key role in regulating digestive functions, immune responses and also influences mental health and cognitive processes, including mood, stress, and memory (Lu et al., 2024). Recent research indicates that dysregulation of this axis may contribute to the development of various neurological and psychiatric disorders, such as depression, anxiety, and Alzheimer's disease (Ashique et al., 2024). Accordingly, increasing attention has been directed towards natural dietary components, particularly plant-derived polysaccharides. Numerous studies indicate that plant-derived polysaccharides possess therapeutic and preventive properties, notably anti-inflammatory and antioxidant activities (Li et al., 2025), along with the promotion of gut health (Zhao et al., 2025). Moreover, earlier findings highlight the health benefits of Plantaginaceae species (Zhang et al., 2020). From the cited findings, the present work aim for the evaluation of the effect of water-soluble polysaccharides from *Plantago albicans* L. on the GBA, using as a model $AlCl_3$ induced AD in Wistar rats.

II.2. Study material

The study materials consist of chemical and biological materials, as well as all the different devices used during the experiment. The biological material includes plant materials and female Wistar rats.

II.2.1. Plant material

For the current study, plant species were collected from the state of El Oued. *Plantago albicans* was found in May 2019 at Debila. Following the collection of plant specimens, the targeted morphological structures, specifically leaves, were isolated. The samples underwent a rigorous cleaning process, initially rinsed with potable water followed by multiple washes with distilled water to eliminate particulate matter and residual contaminants. Post-cleaning, the specimens were subjected to desiccation in a laboratory drying oven maintained at a constant temperature of 40°C. The drying process was monitored gravimetrically, with repeated weight measurements taken until mass

stabilization was achieved, confirming complete dehydration (Samee-Ullah and al., 2019). Subsequently, the desiccated plant material was comminuted using a porcelain mortar and pestle to achieve a homogeneous powder for further analysis.

II.2.2.- Animal material

The study was conducted on 24 female Wistar albino rats at an animal facility at the Faculty of Natural and Life Sciences, University of Chahid Hama Lakhdar El Oued. The rats were weighed (150-170g) and divided into 4 groups, with each group consisting of 6 individuals. They were housed in plastic cages lined with wood shavings at animal laboratory and kept in 12h light /dark room at constant tempertur25 ±3 ^{0}C with a relative humidity of 55± 5. The animals were fed *ad libitium* with standard diet and allowed to adapt for 15 days. Throughout the experiment, the cages were cleaned daily to maintain hygiene. After the adaptation period, the experimental procedures were initiated for all rats.(Al-Hayder et al., 2020).

II.3. Extraction of Water-Soluble Polysaccharides

The extraction of water-soluble polysaccharides from the leaves of *P. albicans* involves several stages, as described below:

Pre-treatment stage: Approximately 112.11 g of dry ground powder is placed in a beaker, and 1120 mL of ethanol (50% v/v) is added at room temperature. The mixture is left for 1.5 hours, covered with aluminum foil, to remove low molecular weight compounds such as polyphenols, fats, and pigments (Tabarsa et al., 2019). The solution is then filtered, and the process is repeated using 560mL of ethanol for 30 minutes. After filtration, 560mL of acetone is added to the solution to further purify the material.

Soaking stage: The pre-treated ground material is soaked in distilled water (110 w/v, approximately 1120 mL) and placed in a water bath at 80 °C for 1.5 hours. This process is repeated at least twice (Fang et al., 2020; Guo et al., 2020).

Concentration stage: The solution is concentrated using a rotary evaporator at 65°C, reducing the volume to approximately 1/3. After completion, the solution is transferred to a tightly sealed bottle and refrigerated overnight at 4 °C to allow further precipitation of impurities. (Sasidharan et al., 2011)

Precipitation stage: The concentrated solution is mixed with 4V of 95% ethanol. Upon adding the ethanol, a layer of organic components precipitates out of the solution. (Tabarsa et al., 2019).

Separation stage: (Centrifugation) The solution is transferred to centrifuge tubes and centrifuged at 3500 rpm for 10 minutes (Lee et al., 2020). The desired precipitate layer is collected, washed with acetone, and dried in a laboratory drying oven. The resulting dry white powder represents the crude extract of water-soluble polysaccharides.

This method ensures the efficient extraction and purification of water-soluble polysaccharides from plant leaves.

The extraction yield is determined according to Guo et al. (2020) as follows:

$$\text{Extraction yield} = \frac{\text{Dry weight of polysaccharide extract (g)}}{\text{Dry weight of the initial plant material (g)}} \times 100$$

II.4. Partial characterization of crude polysaccharide extracts

The components present, including total sugars, neutral sugars, proteins, and polyphenols are determined using colorimetric methods.

II .4.1. Dosage of proteins

Protein quantification is carried out using the method described by Bradford (1976).

Principle: The protein concentration is determined by referencing a standard curve using bovine serum albumin (Bradford, 1976). The reagent used is Coomassie Brilliant Blue, which forms a chromophore complex with a blue color. The resulting coloration reflects the degree of ionisation in the acidic medium, while its intensity corresponds to the concentration of the proteins present (Krim, 2014).

Operating Procedure: A volume of 100 µL of the standard or sample is placed in a dry glass test tube. One (1) mL of Coomassie reagent is added and mixed well, then left to rest for 5 minutes at room temperature. After that, the absorbance is measured at 595 nm. A calibration curve is plotted against the concentration of bovine serum albumin (Bradford, 1976).

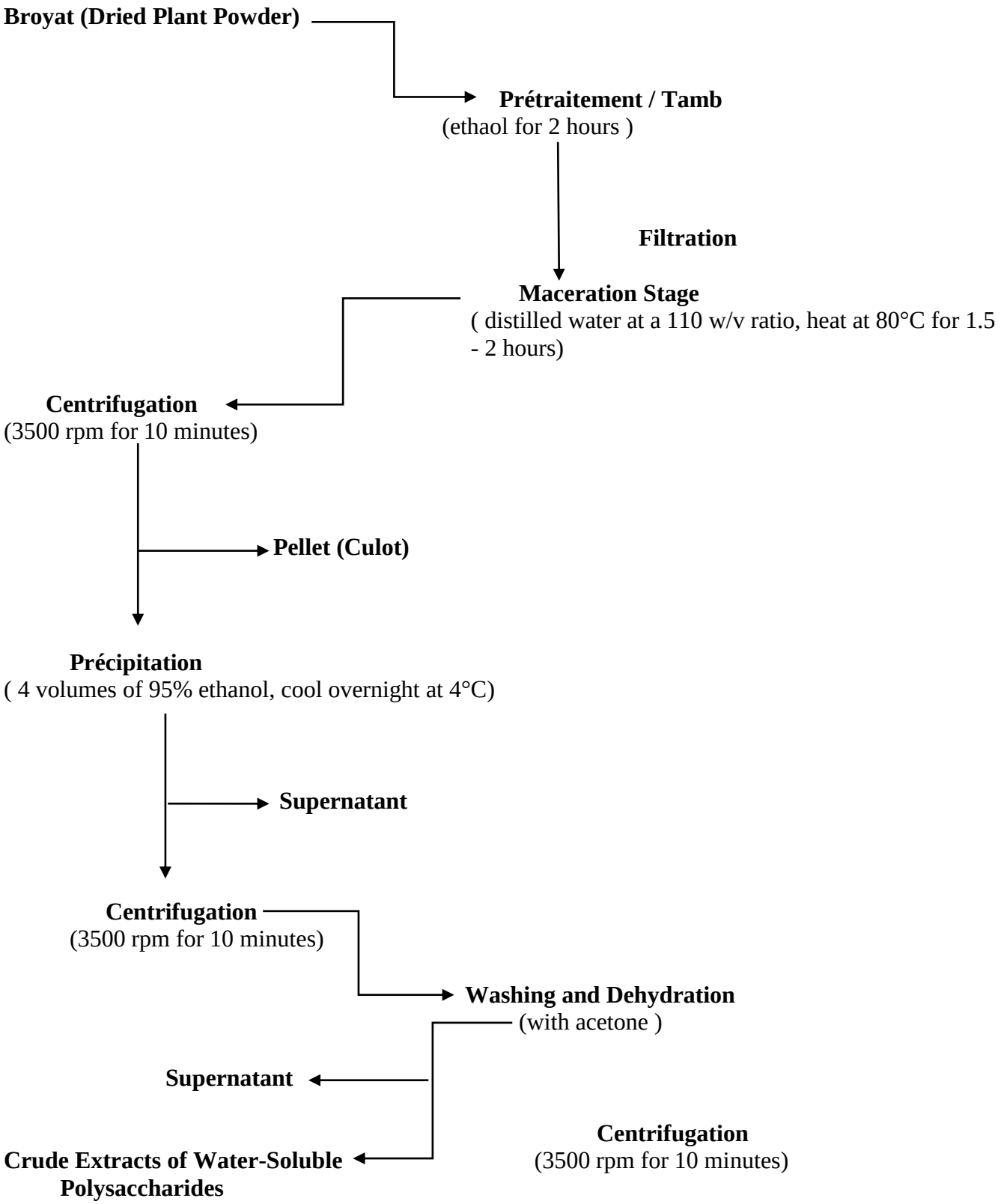

Figure 8: Experimental protocol for water-soluble polysaccharides extraction (Hao et al., 2020; Wang et al., 2014; Kakar et al., 2020; Tabarsa et al., 2018, 2019; Xia et al., 2019)

II.4.2. Dosage of polyphenols

Polyphenols can react with other components. The Folin-Ciocalteu method, described by Singleton and Rossi (1965), was used to determine polyphenols.

Principle: The reagent of Folin-Ciocalteu consists of a mixture of phosphotungstic acid and phosphomolybdic acid. The reduction of the reagent occurs during the oxidation of phenols, resulting in a mixture of blue molybdenum oxides. The resulting color, which has maximum absorption at 765 nm. The color density is proportional to the amount of polyphenols present in the plant extracts (Boiso and Charpentier, 2006). Gallic acid is used as a calibration standard (Slavov et al., 2017).

Operating procedure: A volume of 100 µL of the extract is added to 500 µL of Folin-Ciocalteu reagent diluted to 1/10, then mixed and left for 5 minutes. After that, a volume of 2 mL of a 20% sodium carbonate (Na_2CO_3) is added to the mixture (Amezouar et al., 2013). The measurement of the absorbance is conducted at 765 nm after 30 minutes of incubation at room temperature (Tafinine et al., 2016).

II.4.3. Dosage of total Sugars

The Dubois method (1956) was used to determine the concentration of total sugars in the crude extracts of water-soluble polysaccharides from the studied plant species.

Principle: Simple sugars, oligosaccharides, polysaccharides, and their derivatives produce a yellow-orange color when treated with phenol and concentrated sulphuric acid (Dubois et al., 1956). In the presence of concentrated sulphuric acid and heat, glycosidic bonds are hydrolysed. This is followed by tetradehydration and cyclisation of the released monosaccharides, producing furfural derivatives and 5-formylfuroic acid derivatives. These products condense with phenol to form yellow-orange complexes. The absorbance is measured at 492 nm to determine the total sugar concentration in the sample by referencing a calibration curve, with glucose used as the standard (Brian-Jaisson, 2014; Brudieux, 2007).

Operating procedure: In glass tubes, a volume of 200 µL of the sample or standard is mixed with 200 µL of phenol and 1 mL of concentrated sulphuric acid (H_2SO_4). After incubation of the tubes at 90°C for 5 minutes, a cooling is allowed at room temperature for 30 minutes, with coverage from light. The absorbance is measured at 492 nm (Brudieux, 2007).

II.4.4. Dosage of neutral sugars

The method described by Monsigny et al. (1988) was employed for the quantification of neutral sugars.

Principle: This method is based on the reaction of a concentrated acid with sugars under heat, leading to the formation of furfural derivatives (Dubois et al., 1956).

Operating Procedure: In a test tube, 200 µL of resorcinol (6 mg/mL) and 1 mL of sulphuric acid (98%) are added to 200 µL of the sample or standard. The tubes are thoroughly mixed and heated to 90°C in a water bath for 30 minutes. Subsequently, the tubes are placed in a cold water bath for 30 minutes in the dark. The absorbance of the solution is measured at 480 nm (Monsigny et al., 1988).

II.4.5 Molecular analysis of the sample

Fourier Transform Infrared Spectroscopy (FTIR) was used for the identification of functional groups in chemical compounds by analyzing infrared absorption at different wavelengths.

Principle: The energy of infrared radiation is sufficient to induce molecular vibrations, as all types of chemical bonds respond to this energy by undergoing vibrational movements. As a result, infrared absorption occurs, leading to changes in the dipole moment of the molecule. This absorption indicates that the compound absorbs infrared energy at a specific region of the spectrum (Boukhaloua and Chaouch, 2021).

Operating Procedure: polysaccharide extract from *Plantago albicans* was analyzed using the Cary 630 FTIR spectrometer with an ATR module. The ATR crystal was first cleaned with 70% ethanol and allowed to air dry. A background spectrum was collected prior to sample application.A small amount of the powdered extract was placed directly on the ATR crystal. The pressure arm was gently applied to ensure contact. The IR spectrum was recorded in the range of 4000–500 cm^{-1} using the instrument's software.After measurement, the sample was removed and the crystal was cleaned again. FTIR is a reliable method for identifying functional groups in plant polysaccharides (Sasidharan et al., 2011).

II.5. *In vivo* biological activities

The evaluation of the in vivo effects of polysaccharides is conducted on a model of *W. albino* rats, using aluminum chloride as an inductor of Alzheimer's disease.

II.5.1. Rat treatment

The experimental protocol is proceeded according to Khalil et al. (2020) with some modifications. After a 15-day adaptation period, the experiment was conducted over a period of 35 days following the applied experimental design for the rats. Therefore, a total of 24 *W. albino* female rats were divided into four groups based on their body weight. Each group consisted of six rats and was assigned as follows:

- **Group 1 (Normal):** Received a standard diet and water without any treatment;
- **Group 2 (Negative Control):** Received a standard diet and water along with aluminum chloride ($AlCl_3$) dissolved in water at a dose of 100 mg/kg to induce toxicity;
- **Group 3 (Positive Control) :**Received a standard diet and water, along with aluminum chloride (100 mg/kg) to induce toxicity, and *Plantago albicans L.* polysaccharides extract dissolved in water at a dose of 100 mg/kg;
- **Group 4 (Test) :**Received *Plantago albicans L.* polysaccharides extract alone at a dose of 100 mg/kg, in addition to the standard diet and water.

II.5.2. body weihgt gain

Body weight and its variations were monitored by weighing the rats at the beginning of the experiment (after the adaptation period) and at the end of the experimental period (after 35 days). Weight changes were determined using the following equation (Khalil et al., 2020):

Weight change = Final weight - Initial weight

II.5.3. Behavioral observations

II.5.3.1. Classic labyrinth test

The Classic Labyrinth Test (CLT) is used to evaluate neurobehavioral parameters such as learning ability, and memory in Wistar rats. It also assesses the impact of neurotoxic substances (e.g., drugs, pesticides) on cognition and behavior. The training phase of the test began on Day 30 of the experiment. During the training, bread was used as a guiding stimulus to help the rats navigate the maze and reach the endpoint (Fig.09). On the fifth and final day, recordings were conducted without using bread to eliminate external influences on the rats' behavior. The rats were transferred to the testing room 60 min before the experiment to allow adaptation. The maze was cleaned with 70%

ethanol between trials to remove any scent traces from previous rats. Each rat was placed at the designated starting point. Free exploration was allowed for 5 min. The arrival time is recorded as the time taken to reach the stopping point and remain there for ≥10 seconds (Gasmi et al., 2018).

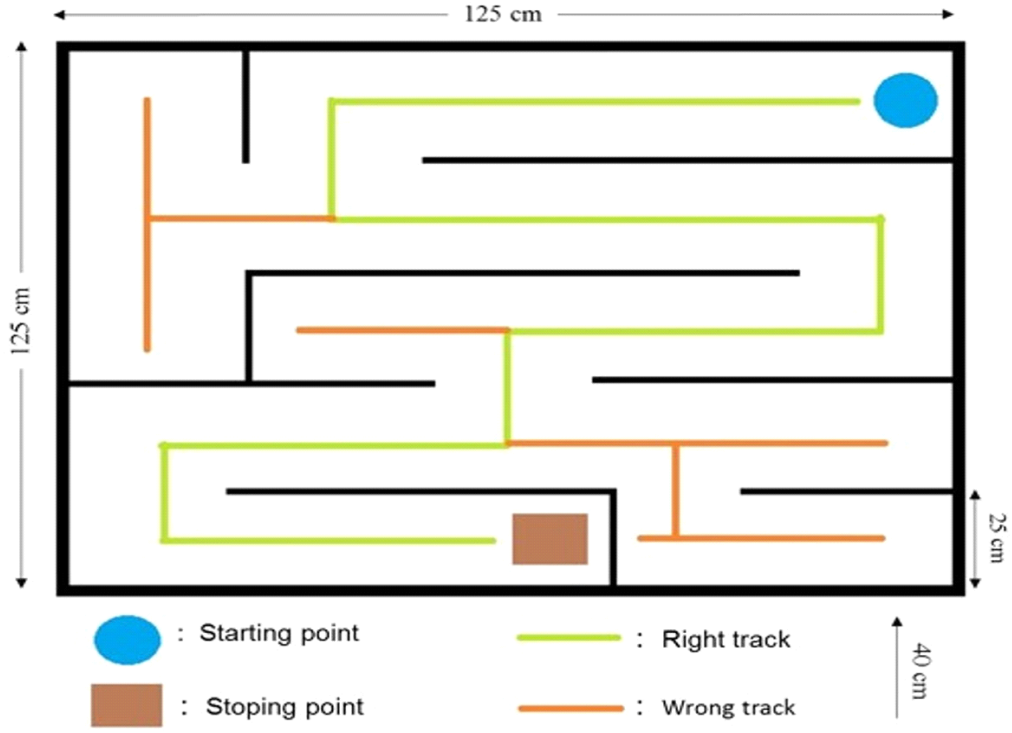

Figure 09: Schematic diagram of the maze layout (Gasmi, 2018)

II.5.3.2. Passive avoidance test

The Passive Avoidance Test help to assess learning and memory retention by analyzing the rodents' avoidance behavior in response to an aversive stimulus. The training phase began on Day 33 of the experiment and lasted two consecutive days. Each rat was placed in the brightly lit compartment, facing the door. Then, the door was opened, allowing the rat to freely enter the dark compartment. Once the rat had fully entered the dark compartment (all four paws inside), the door was closed, and its paws were briefly immersed in cold water as an aversive stimulus. The rat remained in the dark compartment for 10 additional seconds before being returned to its home cage. The apparatus was then cleaned with 70% ethanol before each trial to eliminate residual odors. The retention test was conducted one day after training to evaluate memory retention. Each rat was placed back in the bright compartment, and the door was opened. Latency to re-enter the dark compartment was recorded along with the time that each rat take to leave the dark side, without the application of aversive stimulus. (IACUC, 2021).

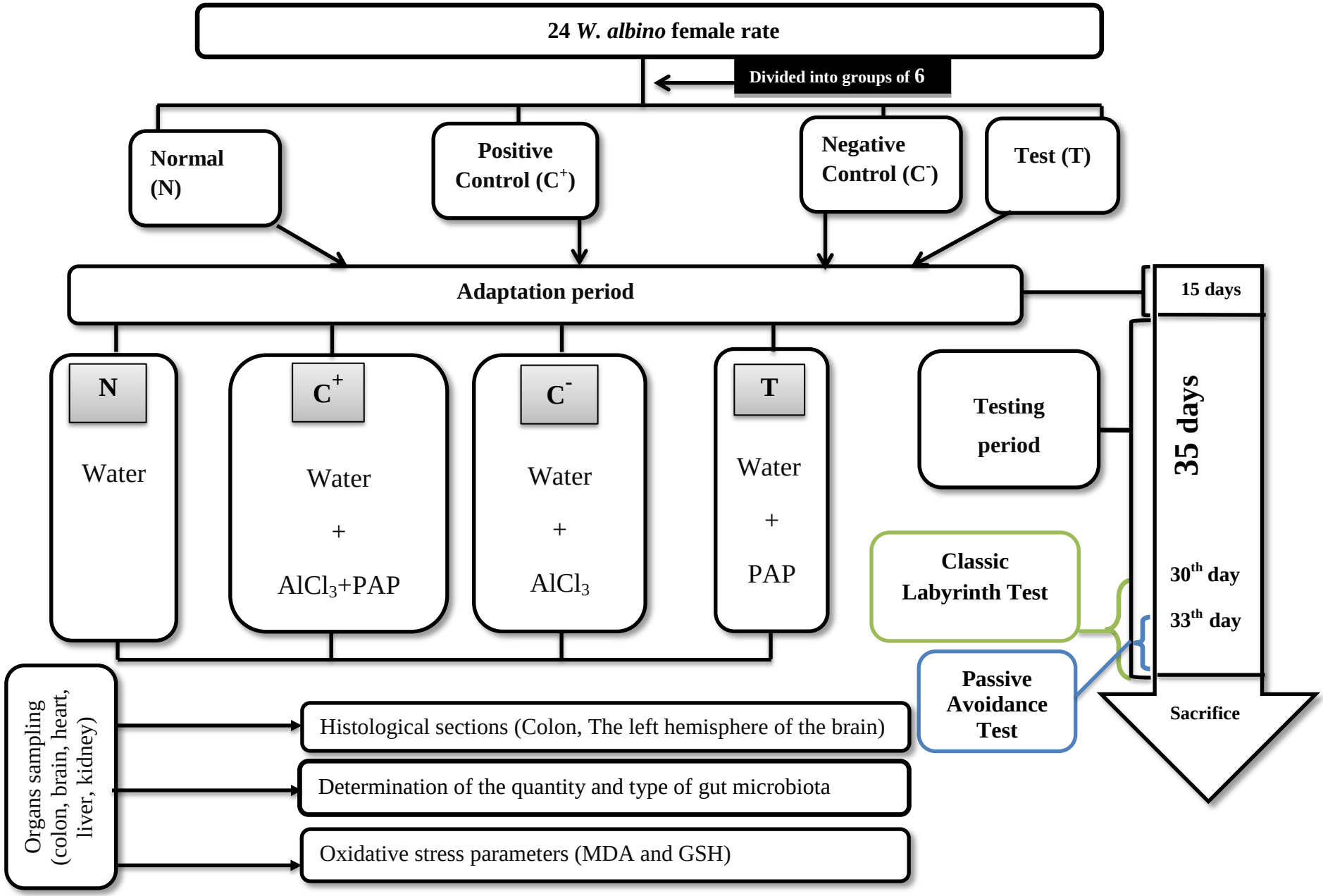

Fig10 The stages of in vivo experimental activity.

II.5.4. Animal sacrifice and samples collection

The rats were sacrificed at the end of the experimental period to collect the necessary samples for analysis according to Derouiche et al. (2018), with some modification. The individuals undergo anesthesia using chloroform (94%) by asphyxiation and are then sacrificed by decapitation. For the histological sections, both colon and right hemisphere of the brain were collected in 10% formalin. In addition, vital organs (brain, kidney, heart, and liver) were collected from all rats in the different groups to evaluate the physiological and pathological effects resulting from the experimentation. According to chasseing et al. (2014), colon swabs were taken from two rats in each group to study gut microbiota.

II.5.5. Oxidative stress parameters

A preparation of organ homogenates (liver, left hemisphere of the brain, heart, and left kidney) is carried out before proceeding with the measurement of oxidative stress parameters.

II.5.5.1. Preparation of organ homogenates

For the preparation of organ homogenates, one gram of each organ is collected and processed. The tissue is ground and homogenised in a Tris-Buffered Saline (TBS) solution, then the resulting cell suspension is centrifuged at 3000 rpm for 10 minutes. The supernatant is then collected, divided into aliquots in Eppendorf tubes, and stored at -4°0C for subsequent analysis of oxidative stress parameters. (Buege & Aust .,1978)

II.5.5 .2. Dosage of malondialdehyde (MDA)

Malondialdehyde (MDA) is one of the final products formed during the breakdown of polyunsaturated fatty acids mediated by free radicals. The level of lipid peroxidation can be assessed by measuring MDA concentration using the TBARS (Thio-Barbituric Acid Reactive Species) test, a commonly used method to indirectly determine lipid peroxidation by quantifying MDA levels in biological samples (Tchantchane et al., 2022).

Principle: This colorimetric method is based on the condensation reaction of malondialdehyde (MDA) in an acidic and high-temperature environment with thiobarbituric acid (TBA). The TBA reaction is a highly sensitive technique that allows the detection of small amounts of lipid peroxides, particularly free MDA. The detection of MDA in biological samples relies on a chemical reaction where two

molecules of TBA react with one molecule of MDA, resulting in the formation of a pink chromogenic complex. The absorbance of this complex is measured using spectrophotometry at 530 nm, where the intensity of the pink color increases with the concentration of MDA. (Buege, & Aust., 1978)

Operating procedure: In tightly sealed glass test tubes, 200 µl of the sample is mixed with 1000 µl of TBA reagent, and the tubes are securely closed. The mixture is then heated in a water bath at 100°C for 15 minutes. After heating, the tubes are cooled in an ice-water bath for 30 minutes while being left open to allow the release of gases formed during the reaction. Following the cooling step, the samples are centrifuged at 3000 rpm for 5 minutes. The absorbance of the supernatant is measured at 532 nm using a UV-Visible spectrophotometer. The TBARS concentration is determined using the molar extinction coefficient of MDA, and the results are expressed in µmol/LOhkawa, (Ohishi& Yagi.,1979).

II.5.5.3 Dosage of reduced glutathione (GSH)

Reduced glutathione (GSH), also known as γ-L-glutamyl-cysteinyl-glycine, is a tripeptide found in all mammalian tissues, with particularly high concentrations in the liver. It plays a crucial role in protecting against oxidative stress, contributes to the detoxification of xenobiotics, regulates the cellular redox status, and influences key biological processes such as cell growth and apoptosis. The quantification of glutathione levels is performed using a standardized method based on the technique developed by Weckbecker and Cory (1988).

Principle: This method is based on measuring the optical absorbance of 2-nitro-5-mercapturic acid, which results from the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the sulfhydryl (-SH) groups of glutathione. To ensure accurate measurement, protein precipitation from the homogenate is essential, allowing for the selective retention of thiol (-SH) groups specific to glutathione for precise quantification. (Tchantchane, al. 2022).

Operating procedure: In a test tube, 800 µl of homogenate is mixed with 200 µl of salicylic acid (0.25%). The mixture is then vortexed and left in the refrigerator at 4°C for 15 minutes.After centrifugation at 1000 rpm for 5 minutes, 500 µl of the supernatant is combined with 1000 µl of Tris buffer (0.4 M Tris, 0.02 M NaCl, pH = 8.9) and 25 µl of DTNB (0.01 M). Following a 5-minute incubation period, the absorbance is measured at 412 nm using a spectrophotometer.

The concentration of GSH, expressed in µmol/mg of tissue(Ellman.,1959), is calculated using the following formula:

$$GSH = \frac{DO \times 1 \times 1.525}{13100 \times 0.8 \times 0.5 \times \text{mg of tissue}}$$

Where:

- DO Optical density (absorbance).
- 1 Total volume of solutions used for deproteinisation (0.8 ml homogenate + 0.2 ml salicylic acid).
- 1.525 Total volume of solutions used in the GSH assay (0.5 ml supernatant + 1 ml Tris + 0.025 ml DTNB).
- 13100 Absorbance coefficient of the -SH group at 412 nm.
- 0.8 Volume of the homogenate.
- 0.5 Volume of the supernatant.

II.6. Histological sections

The preparation of histological sections is carried out using the standard technique established by Houlot (1984), with some modifications according to Bancroft et al. (2012):

Fixing the samples: The samples (colon, brain) were placed in formalin at a concentration of 10% in order to preserve them well, close to the state they were in in the organism, which allows for an optimal view of the tissue structure.

Sample processing and dehydration: Following the fixation step, the samples underwent a series of sequential chemical treatments, including progressive dehydration through graded alcohol solutions, followed by xylene, and finally liquid paraffin. These steps aim to gradually remove water from the tissues and replace it with the embedding medium, ensuring complete tissue saturation with paraffin for precise microscopic sectioning. Before processing, the tissue sections were rotated and dried using an automated system to ensure uniform preparation. This process was carried out using a programmable automated tissue processor of the SLEE (MTP, INS5500GB, 2011) type, ensuring standardized and high-quality tissue processing.

Sample embedding: The tissue samples are immersed in liquid paraffin baths at approximately 70°C, allowing complete tissue impregnation with the embedding medium. Following this step, the embedding process begins, where the paraffin-saturated tissues are placed in molds filled with molten paraffin, which solidifies at -5°C, creating a firm structure that facilitates precise tissue sectioning. This procedure is carried out using a dedicated embedding device equipped with a reservoir containing liquid paraffin of the SLEE (MPS\P1) type.

Preparation of histological sections: The wax block containing the organs is fixed onto the microtome (Thermo Scientific Microm HM325), while the water bath is prepared at 45°C. Sections are then cut at a thickness ranging from 3 to 4 microns. The obtained sections, in which the tissue structure was almost visible, were transferred to the water bath to dissolve the wax, allowing the sample to adhere to the glass slide and drain off excess water. This process was repeated for the remaining wax-embedded samples. The slides were then left to dry overnight in an incubator at 37°C.

Coloration: The Haematoxylin-Eosin (or Hemalun-Eosin) staining technique was applied according to Bancroft et al. (2012) with some modifications. Initially, the sections underwent deparaffinisation through three consecutive xylene baths, remaining for 30 minutes in the first two baths and 15 minutes in the third. Subsequently, the sections were rehydrated gradually by immersion in alcohol solutions with decreasing concentrations (90%, 60%, 30%) for one minute each, followed by thorough rinsing with tap water. The slides were then immersed in a haematoxylin bath for two minutes, which stain the basophilic structures, such as nuclei, in a bluish-purple color. After thorough washing with running water, the sections were stained with eosin by immersing them in an eosin bath for two minutes, resulting in red staining of acidophilic structures, such as the cytoplasm. Following staining, a final rinse with tap water was performed before proceeding with dehydration, achieved by passing the sections through three successive alcohol baths with increasing concentrations (30%, 60%, 90%), for one minute each. Finally, the sections were immersed in two xylene baths for 15 and 5 minutes, respectively, ensuring the complete removal of any remaining solutions.

Microscopic Observation: After drying the samples on the slide and completing the previous staining process, the top glass coverslip was placed and fixed with mounting adhesive. The slides were then ready for microscopic observation using an OPTIKA at 40x 10x magnification.

II.7. Exploration of intestinal microbiota

II.7.1. Determination of the quantity of bacteria

The quantity of bacteria in the colon was identified by using the method of Harley and Prescott (2002), with some modifications. Microbial samples were collected using a sterile swab, applied directly to the cecum mucosa immediately after the sacrifice of the rat. Each swab was immersed in 2 mL of sterile physiological saline (0.9%). The samples were mixed using a vortex to ensure homogeneous dispersion of microorganisms. Afterwards, three specific culture media were prepared for microbiological analysis. Blood agar allows the cultivation of aerobic and facultative anaerobic bacteria, such as Clostridium and Streptococcus. Gelose nutritive (GN) medium (nutrient agar) is used for the general cultivation of microorganisms, including Lactobacilli. The hektoen enteric agar medium (HECT) is **s**elective for enterobacteria**,** particularly E. coli and Salmonella**.** All media were prepared following microbiological standards to ensure sterile and optimal conditions. A volume of 100 µL of the stock solution (raw sample) was transferred into 900 µL of sterile saline solution **to** obtain a 10^{-1} dilution. Also, a volume of 100 µL of the previous dilution was transferred into a new tube containing 900 µL of sterile saline solution to obtain 10^{-2}, 10^{-3}, etc., dilutions**.** After that, 10 µL from each dilution was streaked onto the surface of the culture media using a semi-quantitative method**.** The media were incubated at 37°C for 24 hours under two distinct conditions**:**

- **Aerobic conditions** to promote the growth of aerobic bacteria**.**
- **Anaerobic conditions** in an anaerobic jar to support the growth of strict anaerobic bacteria**.**

II.7.2. Identification of isolated bacteria using the VITEK® 2 compact system

This protocol outlines the steps followed to isolate, cultivate, and identify bacteria present in the samples, using specific culture media and the automated VITEK® 2 Compact system by Idelevich et al. (2018). All procedures were conducted at the El MADJED Medical Analysis Laboratory**.** The diluted samples were inoculated onto the following culture media**:**

- **Blood Agar** For the growth of aerobic and facultative anaerobic bacteria**.**
- **GN (Nutrient Agar)** For the general cultivation of microorganisms.
- **CHROMagar** For the presumptive identification of bacteria based on specific colouration.
- **HECT** For the selection of enterobacteria such as *E. coli* and *Salmonella.*

Petri dishes were incubated for 24 hours at 37°C under aerobic and anaerobic conditions, depending on the bacterial growth requirements. After incubation, colonies were visually examined for size, color, shape, and texture. Colonies of interest were collected using a sterile loop and subcultured onto fresh media for purification**.**

Isolated colonies from different culture media **(**Blood Agar, GN, CHROMagar, and HECT**)** were suspended in sterile saline solution. The bacterial density was adjusted to a specific level **(**0.5–0.63 McFarland**)** using a densitometer. Then, the automated VITEK® 2 compact system enables the precise identification of bacterial species by analyzing their biochemical profiles, including enzymatic activity and sugar fermentation. It was conducted by the insertion of specific cards namely:

GN card Used for gram-negative bacteria. It contains 47 biochemical tests, including:

- Glucose oxidation**.**
- Indole production**.**
- Nitrate reduction**.**
- Urease activity**.**

GP Card Used for Gram-positive bacteria. It includes 43 biochemical tests, such as:

- Arginine dihydrolase activity.
- Alkaline phosphatase activity.
- Acid production from mannitol.
- Pyruvate utilization.

After the GN or GP cards were inserted into the VITEK® 2 Compact system, it analyzed the biochemical reactions and compared the results to an internal database to provide an accurate bacterial identification. The obtained identifications were verified for consistency with initial observations **(**e.g., colony coloration on CHROMagar**).**

II.8. Statistical analysis

The data analysis was performed using XLSTAT (version 2016.02.28451). Results are presented as means ± standard error of the mean (M ± SEM).The Shapiro-Wilk test was used to assess the normality of the data The Kruskal-Wallis test followed by Dunn's test was conducted to identify statistically significant differences. Significance levels were considered at $P \leq 0.05$.

Conclusion and perspectives

Conclusion

In conclusion, this study investigate the relationship between the gastrointestinal system and the brain through the gut-brain axis. The study aims to evaluate the effects of polysaccharides from *P. albicans* on the gut microbiome and its role in alleviating intestinal symptoms and associated neurological effects in aluminum chloride induced Alzheimer's disease. A series of biochemical, behavioral, and microbiological analyses were conducted, allowing for a comprehensive assessment of the extract's multiple effects.

The extracted polysaccharide exhibited a yield of 6.5%, which is considered relatively high compared to similar research findings. Physicochemical analysis showed that PAP is primarily composed of carbohydrates, with high levels of total sugars (72.92% ± 3.65) and neutral sugars (64.53% ± 3.90) as well as a moderate protein and polyphenols content (5.75% ± 1.45, 1.21 ± 0.30 respectively). FTIR spectroscopy confirmed the polysaccharidic nature of PAP through the presence of characteristic absorption bands, particularly a strong peak at 1100 cm^{-1} corresponding to cellulose-type glycosidic linkages.

The results revealed a significant decrease in maze test duration from 188.15 seconds in the C- group to 82.69 seconds in the T group, reflecting an improvement in cognitive and neurological functions after PAP consumption. In the passive avoidance test, the C+ group recorded a time of 145.80 seconds to enter the dark compartment compared to 197.67 seconds in the N group, indicating PAP's positive impact on short- and long-term memory.

Biochemical analyses confirmed a reduction in MDA levels in liver and brain tissues, with the T group recording 0.93 µmol/mg in the liver compared to 1.57 µmol/mg in the C+ group, highlighting PAP's antioxidant effects. On the other hand, the analyses showed a slight increase in GSH levels in kidney tissues in the T group compared to the natural group, suggesting that the extract supports the antioxidant defense system in renal tissues. Furthermore, statistical analyses indicated that the extract's effect on MDA levels in liver tissues was not statistically significant ($p > 0.05$), suggesting that the observed improvement could be due to other factors or that the dose was insufficient to produce a marked effect. Similarly, GSH levels in various tissues did not show significant differences ($p > 0.05$), highlighting the need to reassess the dosage or treatment duration to achieve more notable effects.

Moreover, histological examinations revealed a notable improvement in gut and brain structure, as the mucosal thickness in the C+ group was restored to 567.71 µm, compared to 384.06

µm in the C- group. Neurological analyses showed a reduction in neuronal degradation in the C+ group compared to the negative control group, demonstrating PAP's neuroprotective role against oxidative stress-induced damage.

The results demonstrated that the extract had significant effects on the gut, as evidenced by microbiological analyses that showed an increase in Lactobacillus levels to 1,300,000 CFU/mL in the T group, compared to only 1,000 CFU/mL in the N group, indicating the extract's role in promoting beneficial bacteria.

Based on these findings, the plant extract (PAP) used in this study presents a promising therapeutic alternative for managing intestinal symptoms through its modulatory effects on the gut microbiome and linked neurological functions. This opens new avenues for utilizing plant-based treatments in managing both gastrointestinal and neurological disorders. However, further studies are necessary to determine optimal dosages and mechanisms of action to enhance the extract's efficacy and ensure its long-term stability and safety.

Perspectives

Building on the promising findings of this study, future research should focus on optimizing extraction parameters, including temperature, solvent concentration, and extraction duration, to maximize the yield of bioactive compounds while preserving their structural integrity. Implementing advanced extraction techniques such as supercritical fluid extraction or ultrasound-assisted extraction may further enhance bioavailability and therapeutic efficacy.

Furthermore, to delineate the precise molecular mechanisms underlying the observed effects on gut microbiota and neurological function, comprehensive analyses using advanced techniques such as HPLC and GC-MS are crucial. Nuclear Magnetic Resonance (NMR) spectroscopy could also provide critical insights into the structural composition of the extracted compounds, facilitating the identification of specific bioactive molecules responsible for neuroprotective and anti-inflammatory properties.

Expanding the scope of *in vivo* studies is also essential. Long-term studies involving multiple dosage diets and diverse animal models with both gut and neurodegenerative disorders would help ascertain the optimal therapeutic window and potential side effects. Additionally, examining gut-brain axis interactions through the profiling of neurotransmitters and microbial metabolites would deepen our understanding of the treatment's impact on neurochemical pathways.

Finally, exploring the influence of the extract on other biomarkers, including pro-inflammatory cytokines, oxidative stress markers, and markers of neurogenesis, could open avenues for the development of comprehensive therapeutic strategies targeting both gastrointestinal and neurological disorders. Integrating these findings into clinical studies may also pave the way for novel, plant-based interventions with significant implications for patient care and quality of life.

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Annex

Annex 01 : Colorimetric assays

Protein quantification was carried out according to Bradford (1976) to determine the amount of proteins in the crude extracts using a standard curve (0.01–0.1 g/L) of bovine serum albumin (BSA).

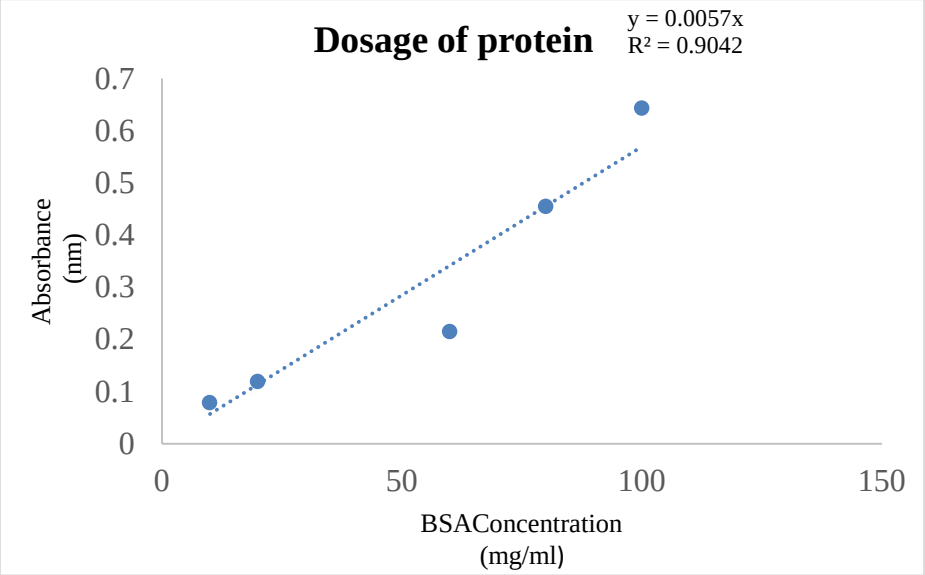


Figure 20:Protein calibration curve

The dosage of polyphenols is carried out by the SINGLETON and ROSSI method (1965) using a range of standards (0.1 – 0.35 mg/l) of gallic acid**.**

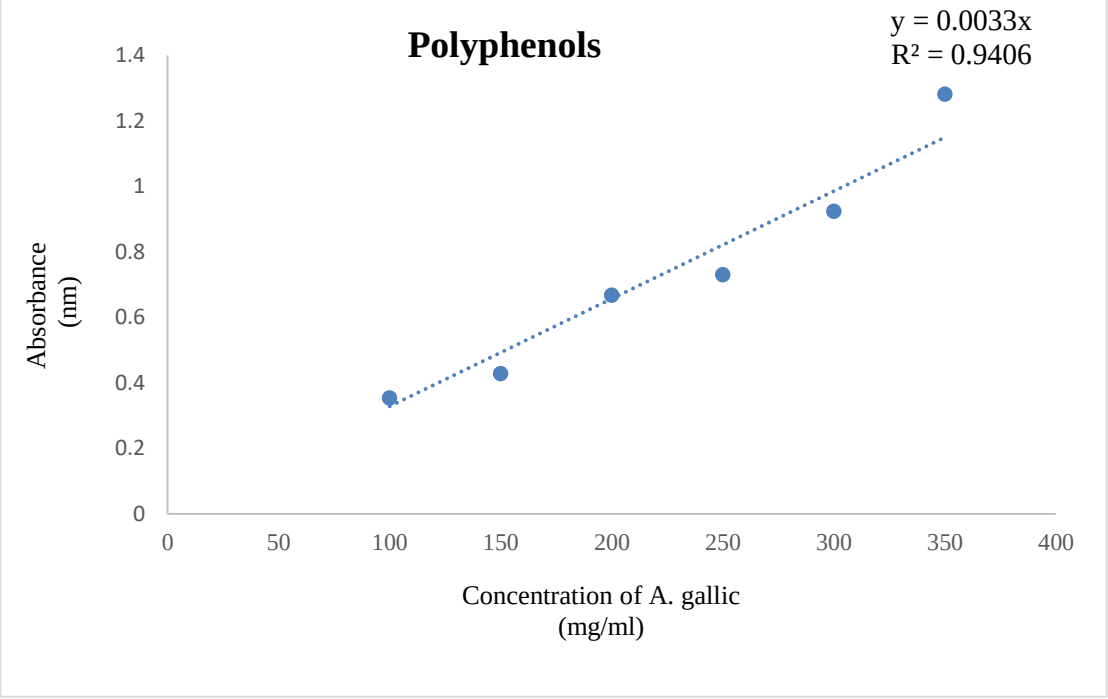


Figure 21 : Calibration curve of polyphenols

The dosage of sugars is carried out by two methods from different concentrations of glucose (Glc) (0.01-0.1 g/l), the total sugars are measured by the Dubois method (1956) and the Monsigny method (1988) made it possible to measure neutral sugars.

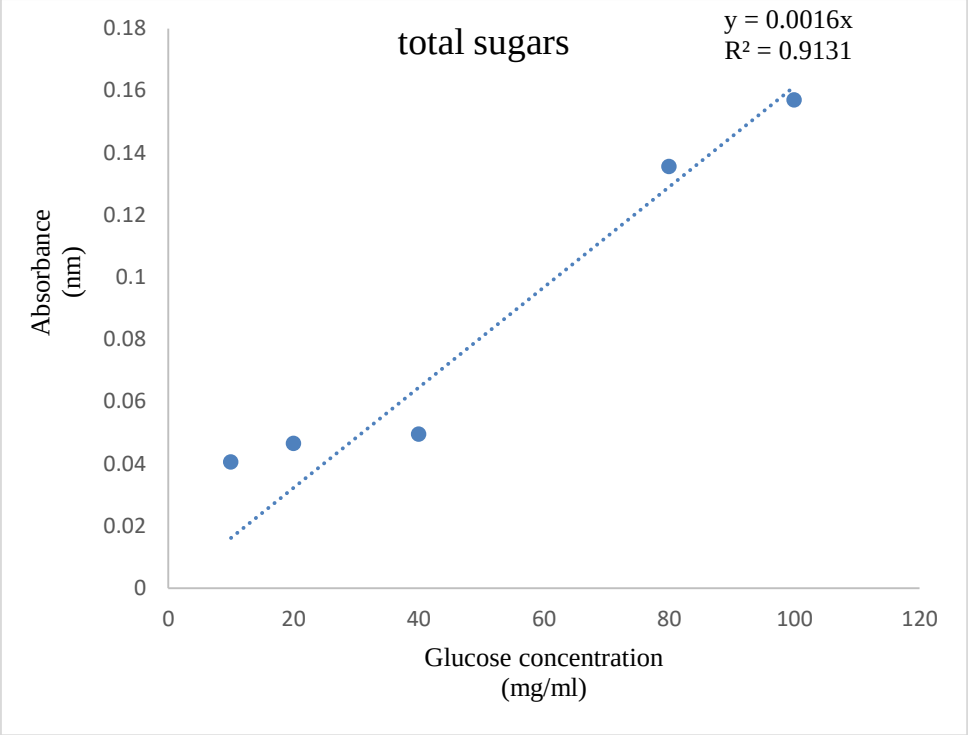


Figure 22 : Calibration curve of total sugars.

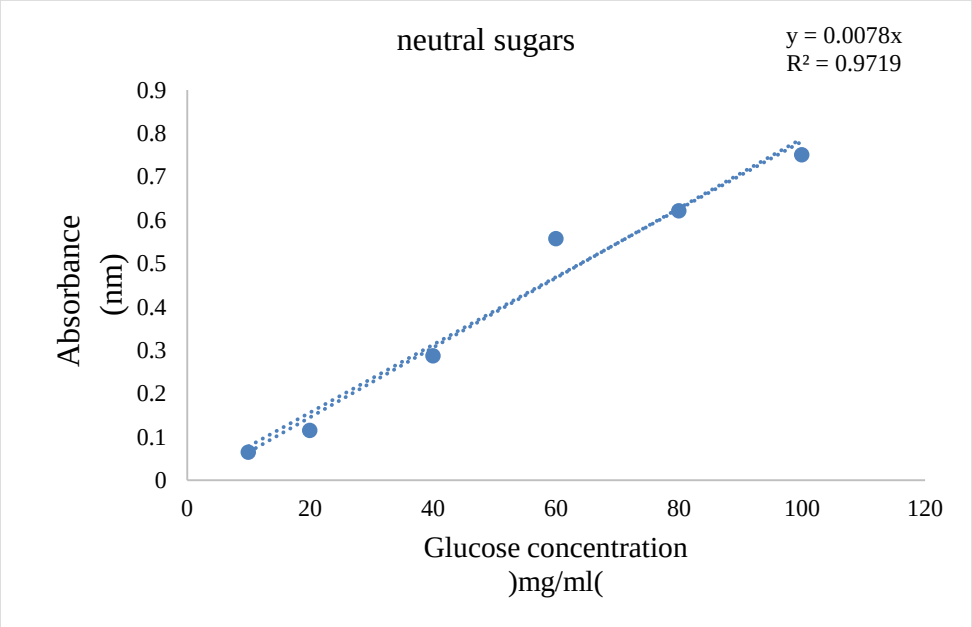


Figure 23Calibration curve of neutral sugars

Annex 02 :Culture media used for bacterial enumeration

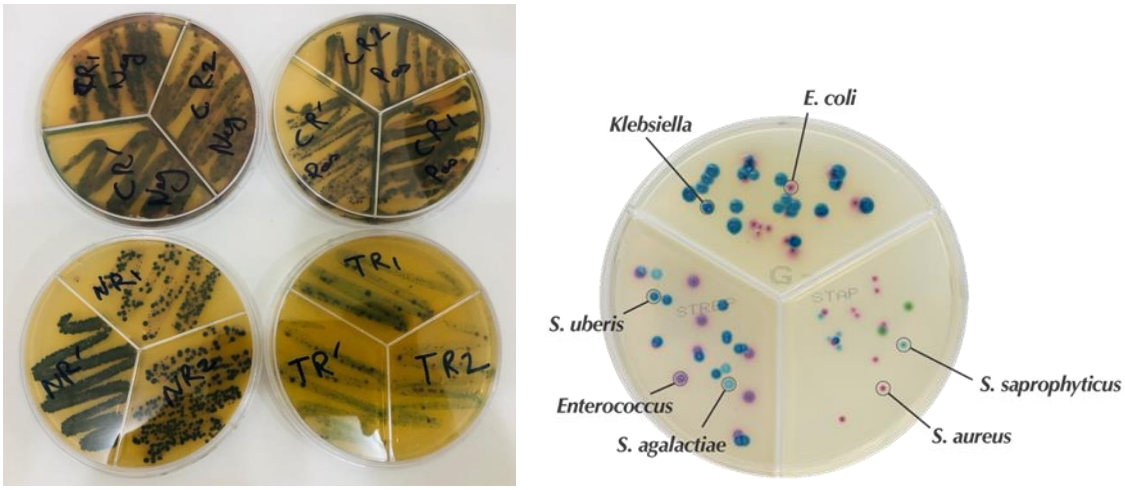


Figure 24 : Sample culture on Chromogenic Orientation Agar for germ identification

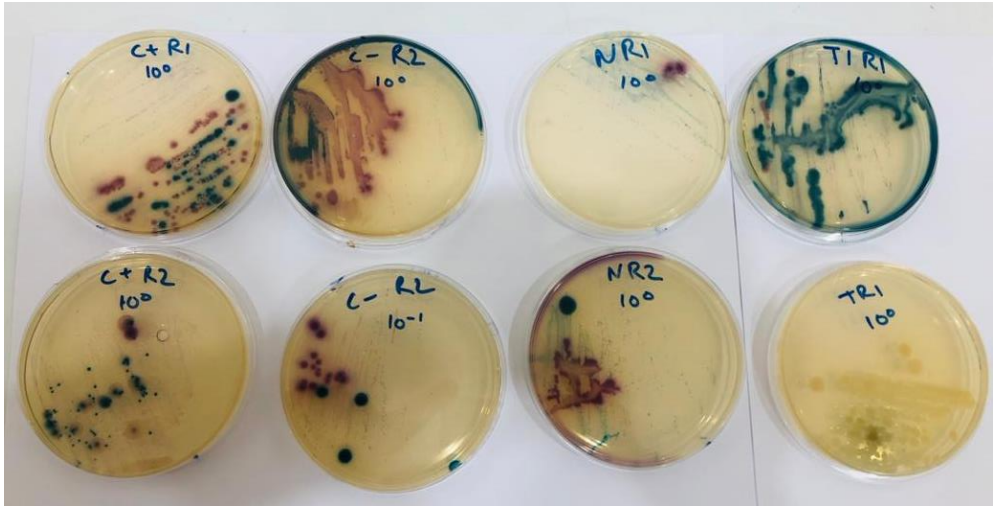


Figure 25 : Sample culture on Chromogenic Orientation Agar under anaerobic conditions for germ enumeration

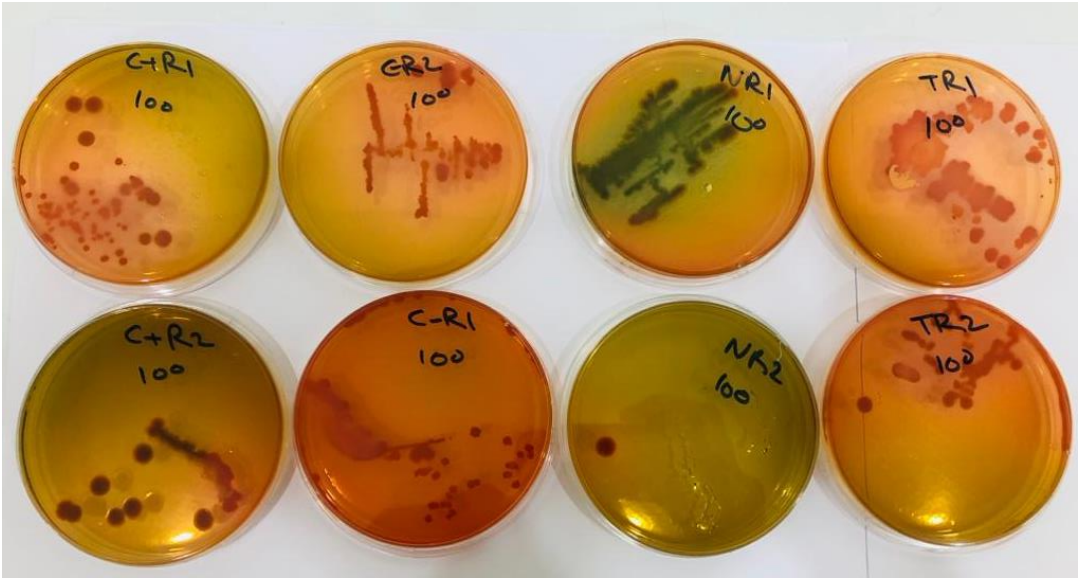


Figure 26: Sample culture on Hektoen agar under aerobic conditions for germ enumeration

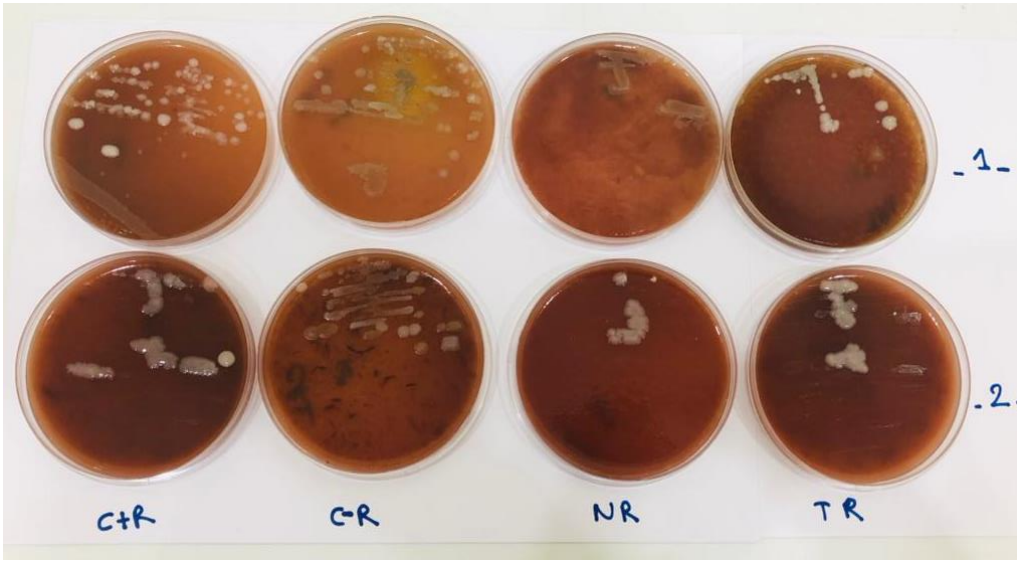


Figure 27 :Sample culture on blood agar under anaerobic conditions for germ enumeration

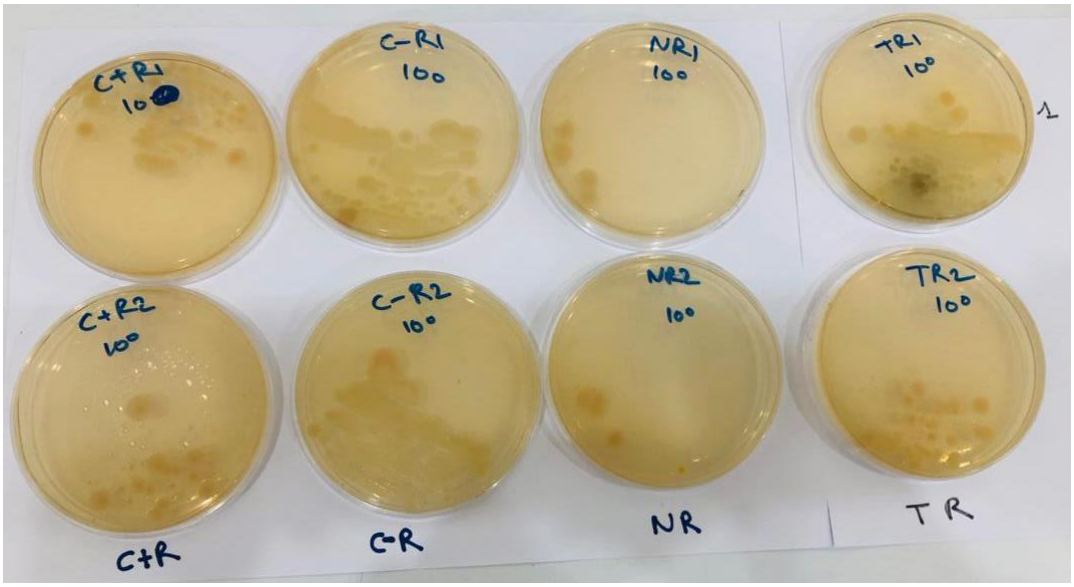
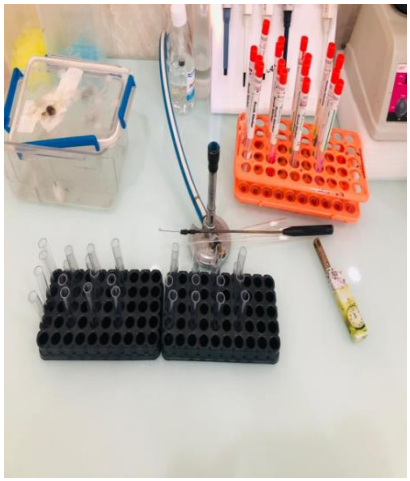


Figure 28 :Sample culture on GN medium under aerobic conditions for germ enumeration

Annex 03 : Procedural steps for bacterial count analysis.



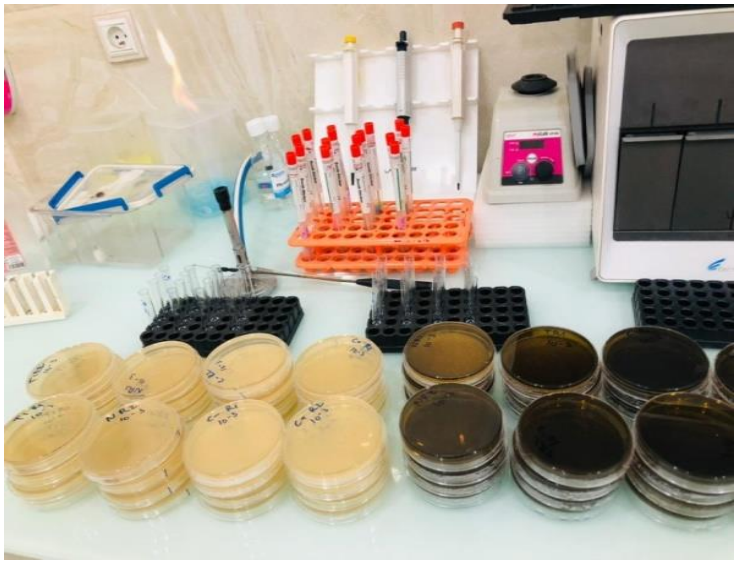


Figure 29: Workbench setup for bacterial enumeration

Annex 04 : VITEK system used for bacterial identification analyses.

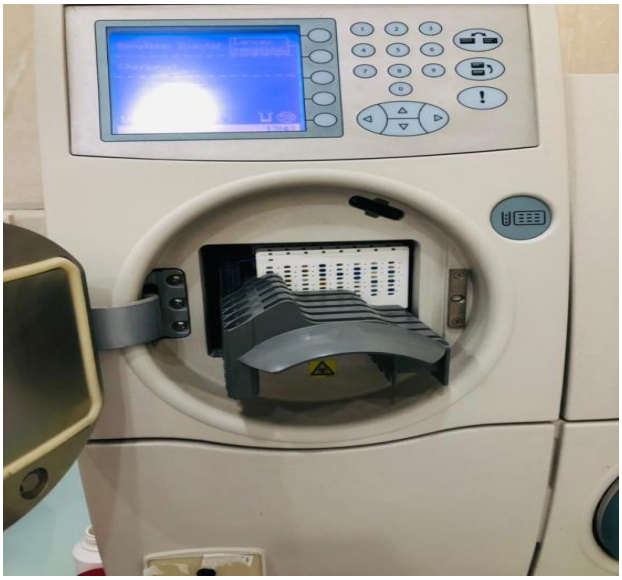


Figure30: Identification of bacteria isolated using the VITEK® 2 Compact system