الجمهورية الجزائرية الديمقراطية الشعبية
République Algérienne Démocratique Et Populaire
وزارة التعليم العالي والبحث العلمي
Ministère De L'enseignement Supérieur Et De La Recherche Scientifique

Université Constantine 1 Frères Mentouri
Faculté des Sciences de la Nature et de la Vie

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Department: Department of Biochemistry and Cellular and Molecular Biology

Thesis presented for the purpose of obtaining the Master's Degree
Field: Natural and Life Sciences
Sector: Biological Sciences
Specialty: Biochemistry

N° d'ordre :
N° de série :

Titled

The modulatory effect of Origanum majorana (Lamiaceae) and Rosa damascena (Rosaceae) on microbiota and redox-state during dysbiosis-mediated food allergy: bibliographic and experimental study.

Presented by: Mrs. Bezziche Mélissa Rania **In :12/06/2024**

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Academic year: 2023 – 2024

Acknowledgements

Thanks…

We would like to thank, first of all, ***Allah*** *who gave us the strength, the flying and the courage to carry out this modest work so as to be able to follow our studies successfully.*

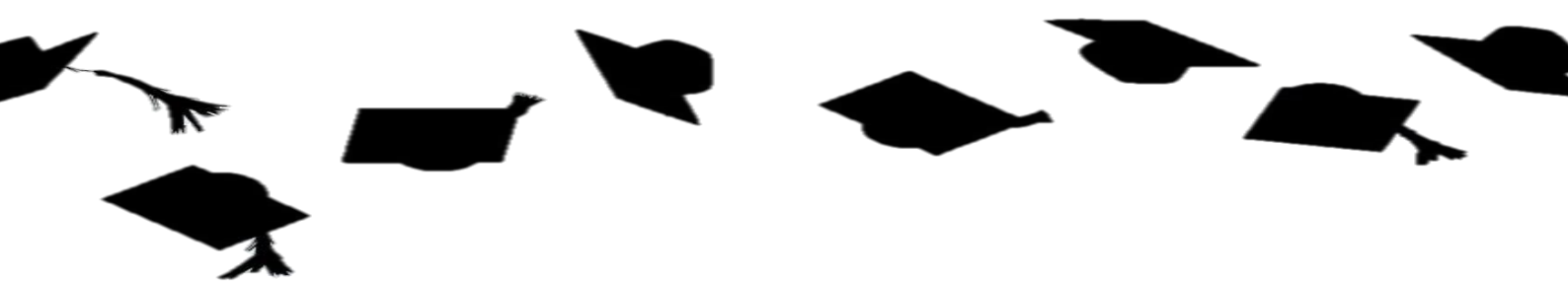
We send our sincere thanks to our coach ***Dr. Ramli Iman*** *who supervised us, for her availability, her wise advice, her encouragement, her kindness, her help we are very honored to work with her.*

To ***Dr. Naouel*** *who has been an ideal lab teacher, mentor and a friend, offering advice and encouragement with a perfect blend of insight and humor. I'm proud and grateful for my time working with her.*

Our thanks are also addressed to the jury members: ***Dr. Bennamoun Leila*** *and* ***Dr. Almi Hiba*** *for having accepted to evaluate and judge our work.*

Finally, we offer our thanks to all those who contributed to the realization of this memory by their knowledge and advice.

Thank you!



Dedicace

With the help of Almighty God, who has given me the path of my life, I have been able to carry out this work that I dedicate:

To my dear and lovely mother: Nouhia Bensebai for her affection, patience, understanding and support throughout my studies, her availability, and her unparalleled support in the most difficult moments of my life. She has showered me with tenderness since my birth with her love and her prayers have always believed in my success.

To my dear father: Nasreddin who always trusted me and pushed me to give the best of myself, through his support, all the sacrifices made and his precious advice. Whatever I do I could not give back to them what they did for me, if I got there it is thanks to them. Nothing in the world is worth the efforts of you day and night for my education and well-being. May God bless them, preserve them, and grant them health and long life. I hope I have fulfilled the hopes you founded in me.

To my only brother: Anis, I love him deeply.

To my dear sister:Djouhina, for their support, I had to go through this dissertation. I love him too much.

To my little nephew: Mohamed Anes Djed, I love him deeply, my affection for you knows no bounds."

To the souls of my beloved Grandmothers "Boulala Malika Maria" and "Boudjarda djamila"

To the souls of my beloved Grandfathers "Abdelmajid" and "taibe"

To my friend: Hafi loubna, Selma Rihane, Darin Boussaha, Sabrina Ghouati I love you.

To all my teachers and all those who have taught me throughout my school life.

To all those who, in a word, gave me the strength to continue and who helped and supported me near or far.

To all those who feel dear to me and whom I have omitted to quote. No dedication can express the love, esteem, dedication and respect I have Always had for you.

♥ Mélissa♥

بسم الله الرحمن الرحيم

قال الله تعالى

"وَقُلِ ٱعْمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمْ وَرَسُولُهُۥ وَٱلْمُؤْمِنُونَ" ۝

صدق الله العظيم

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Abbreviations & Symbols used

IgE	Immunoglobulin E	**APCs**	Antigen-Presenting Cells
RD	*Rosa damascena*	**FcRI**	High-affinity IgE Receptor
IL	Interleukin	**DNA**	Deoxyribonucleic Acid
MHC II -	Major Histocompatibility Complex Class II	**HLA**	Human Leukocyte Antigen
DRB1, DQB1, DPB1	Specific genes within the HLA class II region	**AD**	Atopic Dermatitis
CD14	Cluster of Differentiation 14	**CMA**	Cow's Milk Allergy
SPINK5	Serine Protease Inhibitor Kazal Type 5	**EIF42A**	Eukaryotic Translation Initiation Factor 4A
CD4	Clusters of differentiation	**MetOH**	Methanol.
ZNF281	Zinc Finger Protein 281	**SNPs**	Single Nucleotide Polymorphisms
HTRA2	HtrA Serine Peptidase 2	**OIT**	Oral Immunotherapy
SLIT	Sublingual Immunotherapy	**SCFAs**	Short-Chain Fatty Acids
ILC2	Innate Lymphoid Cell 2	**FMT**	Fecal Microbiota Transplantation
Th0	Naive T Cell	**Th2**	Helper T Cell 2
Treg	T Regulatory Cell	**DPPH**	(2,2-diphenyl-1-picryl-hydrazyl-hydrate
TSLP	Thymic Stromal Lymphopoietin	**sIgA**	Secretory Immunoglobulin A
ROS	Reactive Oxygen Species	**Th2**	Helper T Cell 2
TLR4	Toll-like Receptor 4	**GAE**	Gallic acid equivalent
WHO	World Health Organization	**E.coli**	Escherichia coli
S. aureus	*Staphylococcus aureus*	**OM**	*Origanum majorana.L*
ATCC	American type culture collection		

Introduction

The utilization of natural products for addressing health issues has been a longstanding practice throughout human history, representing one of the earliest forms of empirical treatments. Medicinal plants have been extensively used in developing countries, including Brazil, which has witnessed a growth in alternative medicine such as herbal medicine. This branch of medicine offers diverse treatment options for various types of ailments.

Food allergy is defined as an immune reaction to proteins in the food and can be immunoglobulin (Ig)E-mediated or non-IgE-mediated. IgE-mediated food allergy is a worldwide health problem that affects millions of persons and numerous aspects of a person's (Lopez, Yarrarapu, et Mendez 2024).

Gut microbiota refers to the diverse community of microbes residing in the gut, including bacteria, fungi, viruses, and parasites. A balanced microbiota supports gut health by promoting enterocyte function, maintaining the integrity of the intestinal barrier, preventing the adhesion of pathogenic bacteria, and producing essential vitamins. Conversely, disruptions to the microbiota, known as dysbiosis, can lead to various diseases (Liang et al. 2023) . Emerging evidence highlights the significance of gut microbiota in the development of food allergies. They help maintain the intestinal barrier and modulate gut inflammation.

Medications commonly used to treat food allergies can cause adverse effects, prompting the search for natural and safer alternatives. Interventions that modify the gut microbiota, such as prebiotics, probiotics, and fecal microbiota transplantation (FMT), are being a prominsing novel treatments for conditions with increasing prevalence and social impact(Chaves et al. 2020). Research indicates that herbal medicines can help modulate immune responses, making them a potential candidate for allergy treatment (Chaves et al. 2020).

Origanum majorana, and *Rosa damascena*, are two plants that have been traditionally used for their aromatic and medicinal properties Recent studies have investigated their potential roles in managing food allergies and influencing gut microbiota, areas of significant interest given the increasing prevalence of allergic diseases and the critical role of microbiota in human health (Petersen et Simmonds 2003).

Origanum majorana has been studied for its anti-inflammatory and antioxidant properties, which can help modulate immune responses and reduce allergic reactions. Its bioactive compounds, such as flavonoids and phenolic acids, contribute to its therapeutic(Sienkiewicz et al. 2011). Marjoram can influence gut microbiota by promoting the growth of beneficial

bacteria, enhancing gut health, and potentially alleviating food allergies through its immunomodulatory effects (Aytaç 2020).

Rosa damascena is known for its anti-inflammatory, antioxidant, and antimicrobial properties. These properties help in maintaining the integrity of the gut barrier and modulating the gut microbiota composition. By reducing gut inflammation and supporting a balanced microbiota, Rosa damascena may help mitigate food allergies. Its essential oils and phenolic compounds are particularly effective in promoting gut health and reducing allergic symptoms (Shukla et Gupta 2010).

Both O*riganum majorana* and *Rosa damascena* offer promising benefits in the context of food allergies and gut microbiota health. Their bioactive compounds and effects on inflammation, immunity, and microbial balance provide a foundation for further research. Integrating these plants into the diet could potentially offer complementary strategies for managing allergies and promoting a healthy gut microbiota, contributing to better overall health outcomes.

Origanum majorana and Rosa *damascena* were selected for this study on food allergies and gut microbiota due to their unique and beneficial properties is known for its anti-inflammatory, antioxidant, and antimicrobial effects, this plant can influence gut health by modulating the gut microbiota, potentially reducing allergic responses.

The primary objective of this study is to investigate the beneficial activities of various extracts from *Origanum majorana* and *Rosa damascena* and to provide comprehensive insights into the phenolic and flavonoid composition, antioxidant, and antibacterial activities of *Origanum majorana* and *Rosa damascena* extracts by:

- Investigating the phenolic profile composition through phenolic and flavonoid content analysis, Determine the types and quantities of phenolic compounds and flavonoids in the extracts, These compounds can affect gut microbiota composition by promoting beneficial bacteria and inhibiting pathogenic strains and May influence the immune response and inflammation, potentially impacting food allergy symptoms.
- Testing the in vitro antioxidant activity, Assess the ability of extracts to neutralize free radicals or prevent oxidation.
- Evaluating the antibacterial activity of the extracts, Determine the effectiveness of extracts against various bacterial strains.

Bibliographic study

Chapter one:

Food allergy and dysbiosis

I. Food allergy

Food allergy is an immune system reaction that occurs shortly after consuming a particular food. When someone has a food allergy, their immune system mistakenly identifies certain proteins in the food as harmful, triggering the production of antibodies to fight them off. This immune response can lead to various symptoms ranging from mild to severe, such as hives, itching, swelling, difficulty breathing, abdominal pain, diarrhea, or even life-threatening anaphylaxis It's important for individuals with food allergies to carefully manage their diet and avoid the specific foods that trigger their allergic reactions (Imane 2021).

I.1 Immunopathology of food allergy

Food allergy is typically caused by IgE and is associated with type I hypersensitivity. However, there are other types of food allergies independent of IgE and represents approximately 10% of cases (Pascal 2011). They are linked to other categories of hypersensitivity, such as type III (complex immune) or type IV (delayed hypersensitivity) Food allergies are not limited to the effects of trophallergen ingestion, which are allergens of food origin. Although it is the most documented route and best demonstrated, there are cases where allergens can enter through the system respiratory during cooking or by simple skin contact. Although many food proteins can be allergens, 90% of Food allergies are caused by a few foods (Rayan 2021).

I.2 Etiology

The etiology of food allergies is intricate and encompasses a blend of genetic predisposition, immunological responses, environmental factors, and early life experiences. Genetic predisposition plays a significant role, as individuals with a family history of allergies are more susceptible to developing food allergies themselves. Immunologically, food allergies occur when the immune system erroneously identifies harmless food proteins as harmful invaders, prompting the production of antibodies, notably Immunoglobulin E (IgE). Subsequent exposures to the allergenic food trigger the release of histamine and other chemicals, instigating allergic symptoms (Figure 01) (Laoubi 2020).

Environmental elements also influence the development of food allergies. Early exposure to allergenic foods, either through breastfeeding or the introduction of solid foods, may impact immune responses.

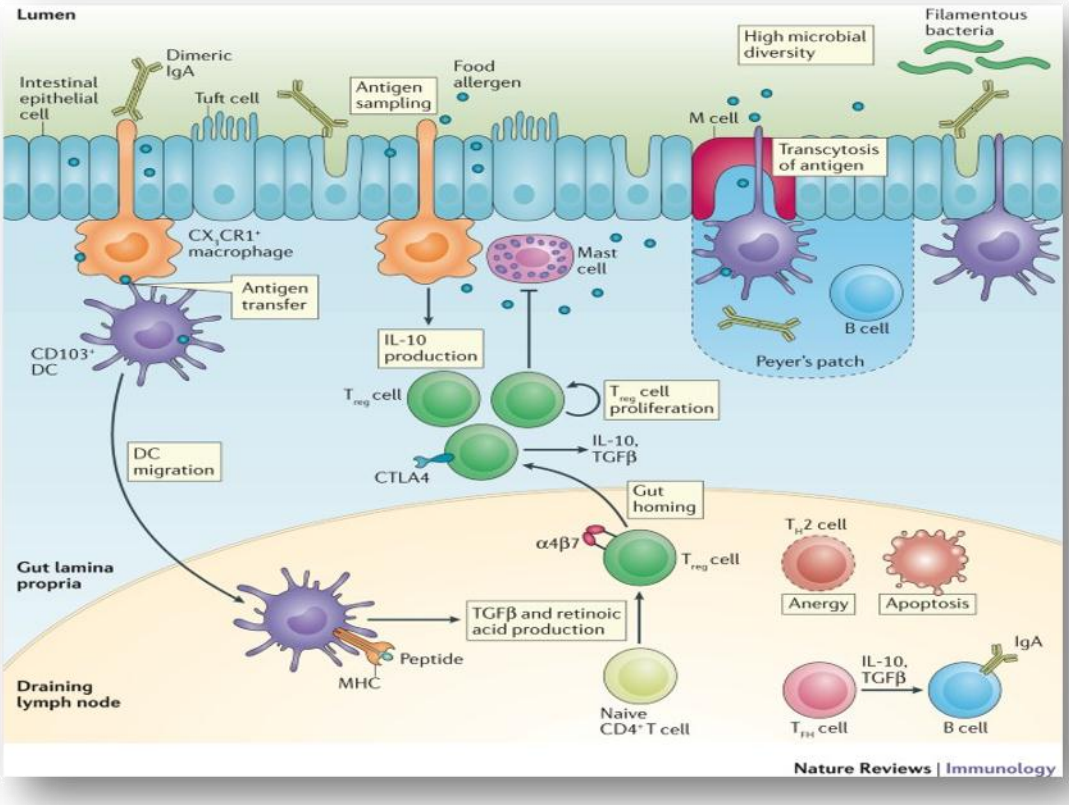

Figure 01. Food allergy immune mechanisms (Wong 2016).

Moreover, environmental allergens, such as pollen or dust mites, can contribute to cross-reactivity, where proteins in different allergens share structural similarities, leading to allergic reactions. The hygiene hypothesis suggests that reduced exposure to microbial diversity and infectious agents in early childhood could increase allergy risk, emphasizing the crucial role of early immune system development (Ouahiba et Khawla 2023)

Furthermore, disruptions in gut microbiota balance, known as dysbiosis, are implicated in food allergy development by influencing immune function and intestinal permeability. Collectively, these factors interact intricately, shaping the complex etiology of food allergies. Ongoing research endeavors strive to unravel the mechanisms underlying food allergies and devise effective strategies for prevention and treatment in clinical settings (Ouahiba et Khawla 2023).

I.3 Pathophysiology

Food allergy occurs most often in people with atopy characterized by high production of IgE in response to an allergic stimulus. On the other hand, Some individuals who self-identify as allergic may not truly have allergies. This discrepancy can arise due to several factors (Vickery, Chin, et Burks 2011). Firstly, symptoms commonly associated with allergies, such as nasal

congestion or skin irritation, can be caused by various non-allergic factors like irritants or environmental conditions. Secondly, psychological elements can play a role, with individuals experiencing symptoms due to stress or anxiety rather than an actual allergic reaction. Lastly, underlying medical conditions unrelated to allergies, such as sinus infections or respiratory issues, can manifest symptoms similar to those of allergies. Thus, while individuals may believe they have allergies, a comprehensive medical assessment is essential to accurately diagnose and address their symptoms (Pelz et Bryce 2015). The mechanism takes place in two distinct phases: awareness phase and awareness phase triggering (Fischler 2013).

- First step: the awareness phase.

During allergic sensitization, antigen-presenting cells of gut-associated lymphoid tissue take up food antigens and, after intracellular cleavage, bind the antigen-derived peptides to MHC II molecules on their surface(Figure 02). The APCs thus activated then migrate to the "local" lymph nodes where they interact with naive CD4+ T lymphocytes (Fischler 2013).

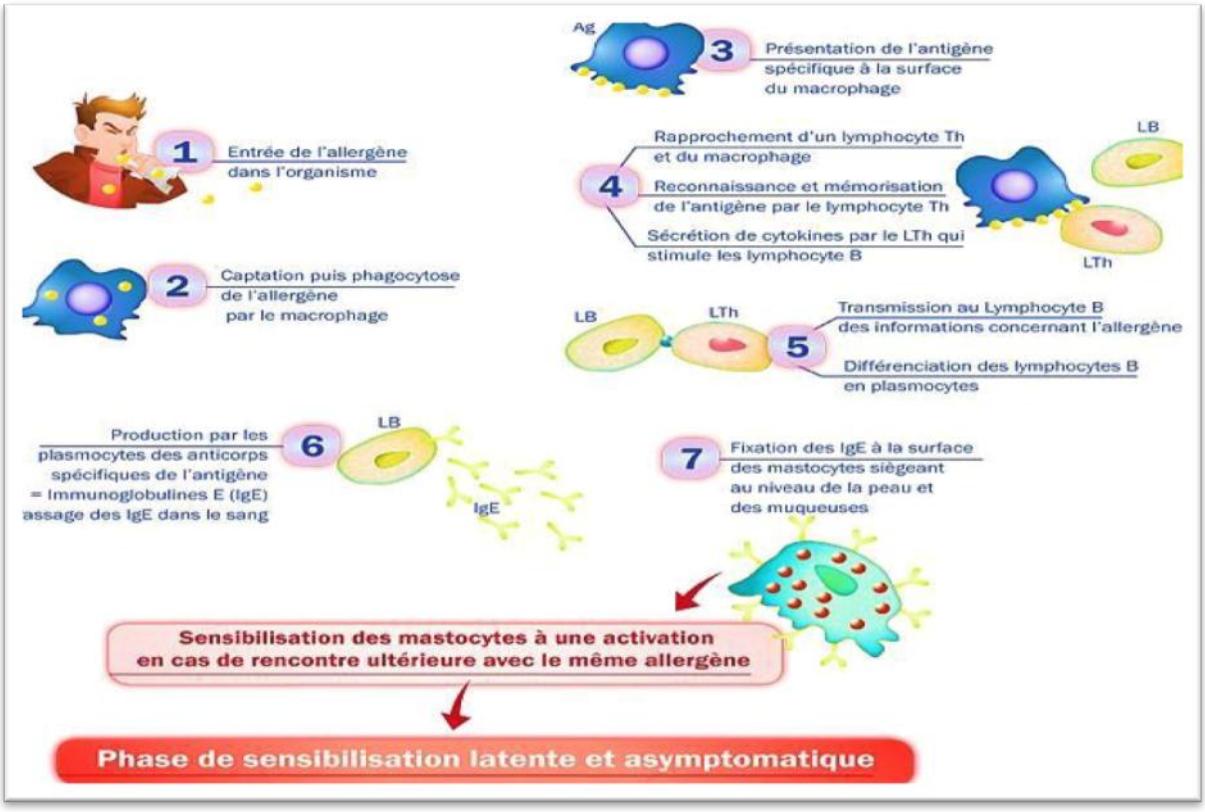

Figure 02. Allergic sensitization phase (first contact)(Berkani et Lallali 2020)

- Second stage: Trigger phase:

During the second contact of the allergen with the body, it is recognized by specific IgE bound to effector cells via receptors. IgE bridging will result in aggregation of FcRI, which will induce phosphorylation of tyrosine residues present on the receptor. At the start of an allergic reaction, there are two phases, shown schematically in (Figure 03): The early phase is very rapid, mainly due to the direct effect of the released histamine, which acts on the levels of H1 receptors in the blood vessels and bronchi. The late phase begins within two to eight hours after threshing and lasts at least one to two days (Imane 2021).

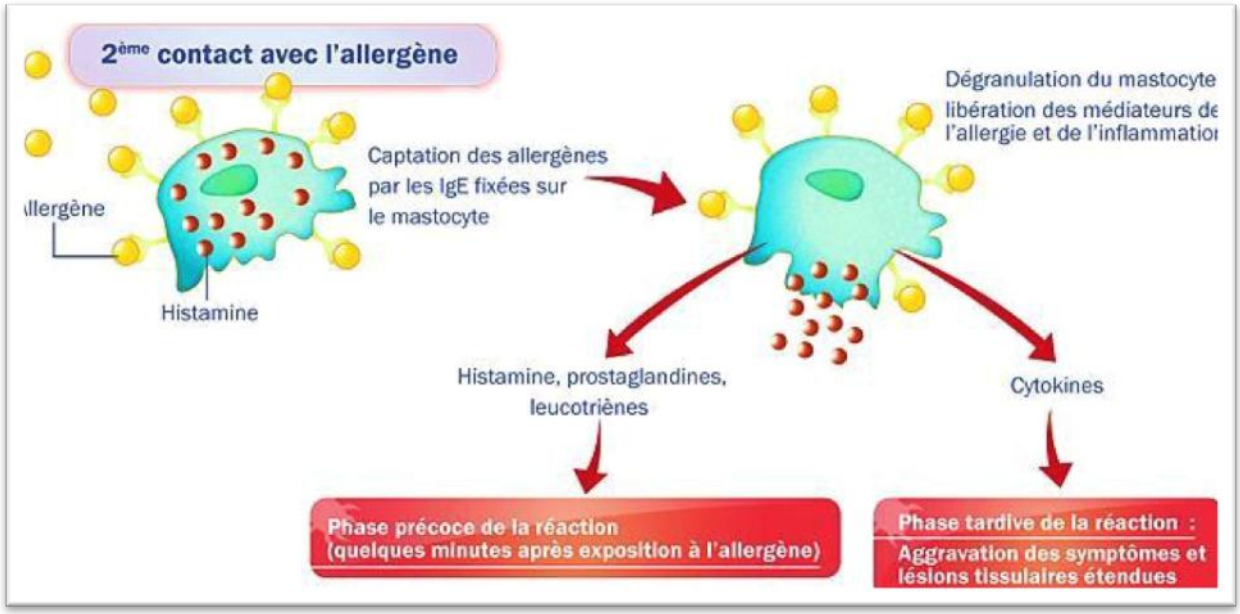

Figure 03. The triggering phase (second contact) (Berkani et Lallali 2020).

I.4 Genetics of food allergy

Various experimental designs and analytical approaches, such as family studies, twin studies, cohort studies, and methods like linkage analysis, candidate gene analysis, genome-wide association studies, DNA methylation analysis, and microbiome analysis, have been employed to address key questions. These include understanding why some individuals develop food allergies while others do not, and how genetic and environmental factors interact to increase the risk of food allergies. Researchers screen the genome to identify regions contributing to variance in food allergy-related outcomes, allowing for a comprehensive understanding of the genetic underpinnings of food allergies and potential avenues for intervention (Johansson et Mersha 2021).

- **Major Histocompatibility Complex (HLA) Gene Family**

Previous studies have reported significant associations between peanut allergy and polymorphisms in the HLA class II genes: DRB1, DQB1, and DPB1 (Howell et al. 1998).

- **CD14 Gene**

The CD14 gene, which encodes the receptor for lipopolysaccharides, is also of interest in food allergy research. In a study involving 175asthmatic and 77 food-allergic patients of various ages, the C-159T polymorphism in the promoter region of CD14 was found to be associated with non-atopic asthma and food allergies, especially among white subjects (Woo et al. 2003)

- **FOXP3 Gene**

Forkhead box P3 (FOXP3) is considered the best marker for naturally occurring regulatory T-cells. Torgerson et al. reported that a 1300-base pair deletion in the non-coding region of the FOXP3 gene can result in reduced FOXP3 mRNA levels and significantly decreased protein expression in peripheral blood lymphocytes(Woo et al. 2003).

- **STAT6 Gene**

Signal transducer and activator of transcription 6 (STAT6) plays a crucial role in the IL-4 and IL-13 signaling pathway, which is involved in IgE isotype switching and TH2 cytokine production. Amoli et al. reported that the G allele of the STAT6 G2964A polymorphism was significantly more common in nut-allergic patients compared to controls, following a recessive model (Johansson et Mersha 2021).

- **SPINK5 Gene**

Serine protease inhibitor Kazal type 5 (SPINK5) is a protease inhibitor protein that is expressed in the thymus. Defects in SPINK5 have been linked to abnormal T lymphocyte maturation, leading to Th2 responses, increased IgE levels, and eosinophilia (Johansson et Mersha 2021).

- **Interleukin 10 (IL10) Gene**

IL10 down-regulates Th1 cytokines, MHC class II antigens, and costimulatory molecules on macrophages. Two SNPs, A-1082G and C-627A, in the IL10 gene are associated with its production. Negoro et al. found that the IL10 -627AA polymorphism was significantly linked to the severity of food allergy (FA) and atopic dermatitis (AD) in 220 Japanese allergic children. However, a recent study of atopic Japanese children found no association between IL10 -627AA and the prevalence of FA. Instead, they reported that children with the IL10 -1082AA genotype had a significantly higher risk of FA, with a 2.5 times increase (Johansson et Mersha 2021).

- **Interleukin 13 (IL13) Gene**

IL13, an important immunoregulatory cytokine produced mainly by activated Th2 cells, has gene polymorphisms that have been linked to asthma in over 25 studies. A recent multicenter study of unrelated German children found that the C-1055T polymorphism in the IL13 gene is associated with an increased risk of food sensitization (Johansson et Mersha 2021).

I.5 Epigenetics of food allergy

Recent studies have revealed that epigenetics and environmental exposures contribute to the development of food allergies, and reveal new mechanistic insights into disease. In this section, we will describe the most recent evidences on the epigenetic modifications in the most frequent food allergies. For instance, studies have uncovered epigenetic changes related to DNA methylation in regulating genes involved in food allergies (Woo et al. 2003).

Specifically, DNA methylation of cytokine genes IFN-γ, IL-4, and IL-5, as well as the FOXP3 gene, has been shown to influence food allergies. Additionally, miR-193a-5p has been identified as a regulator of IL-4 gene expression, potentially impacting IgE-mediated food allergies. Moreover, researchers have observed distinct DNA methylation patterns in Th1 and Th2 cytokine genes and epigenetic regulation of the FOXP3 gene in children acquiring tolerance to IgE-mediated food allergies. This suggests that epigenetic mechanisms play a role in the development of tolerance in these individuals (Woo et al. 2003).

In another pilot study on food allergies, hypermethylation was found in specific gene regions (DHX58, ZNF281, EIF42A, and HTRA2) in food allergies patients compared to healthy controls. Interestingly, this hypermethylation pattern was not observed in tolerant food allergies patients, indicating a potential association between DNA methylation patterns and tolerance to proteins in food allergies patients (Woo et al. 2003).

I.6 Epidemiology of food allergy

The epidemiology of food allergies varies globally, with prevalence rates influenced by genetic, environmental, cultural, and dietary factors. Globally, estimates suggest that approximately 2-10% of the population is affected by food allergies, with common allergens including peanuts, tree nuts, milk, eggs, soy, wheat, fish, and shellfish. However, data on food allergies in Africa are relatively limited. While some studies indicate that food allergies may be less prevalent in

African countries compared to Western nations, factors such as changes in lifestyle, urbanization, and dietary habits may impact prevalence rates (Sicherer et Sampson 2009).

Specific data on food allergies in Algeria, a North African country, are scarce. Allergic diseases, including food allergies, have received less attention compared to other regions. Algeria's diverse cuisine, which includes ingredients like nuts, dairy, wheat, and seafood, may contribute to the prevalence of food allergies. However, limited awareness, access to healthcare, and diagnostic resources may hinder the recognition and reporting of food allergies in Algeria (Bartha, Almulhem, et Santos 2023).

I.7 Mechanisms of Allergic Reactions and Current immunotherapeutic approaches for food Allergy

Food allergy involves an acute hypersensitivity reaction caused by IgE antibodies produced in response to specific food allergens. This hypersensitivity can provoke systemic or localized inflammatory responses, manifesting as swelling, urticaria, eczema, airway hyper-responsiveness, asthma, and potentially leading to a life-threatening severe systemic reaction known as anaphylaxis (Kunal 2020). A comprehensive overview of the mechanism of a typical type-I hypersensitivity reaction is presented in (Figure 4).

I.8 Symptoms

Individuals with allergies, ingestion of food can cause rapid onset of symptoms often within a few minutes or less than an hour, these symptoms get worse generally over time (Laoubi 2020). The coccurence of these symptoms also depends on different parameters: quantity ingested, other compounds ingested, possible transformation of foods and absorption rates (Seth et al. 2020). These symptoms can also affect the digestive, respiratory, cardiovascular or endocrine systems, or they can be systemic (Laoubi 2020).

Common symptoms in food allergy can be manifested as: anaphylactic shock and acute anaphylaxis. (Turner et al. 2022), skin signs, hives and swelling of the Quincke, Systemic (malaise), respiratory (dyspnea, bronchospasm), cardiovascular problems (hypotension, tachycardia) (Turner et al. 2022), digestive symptoms such as late diarrhea (Inès 2023), symptoms hyperexcretion at the ocular, nasal, bronchial level.

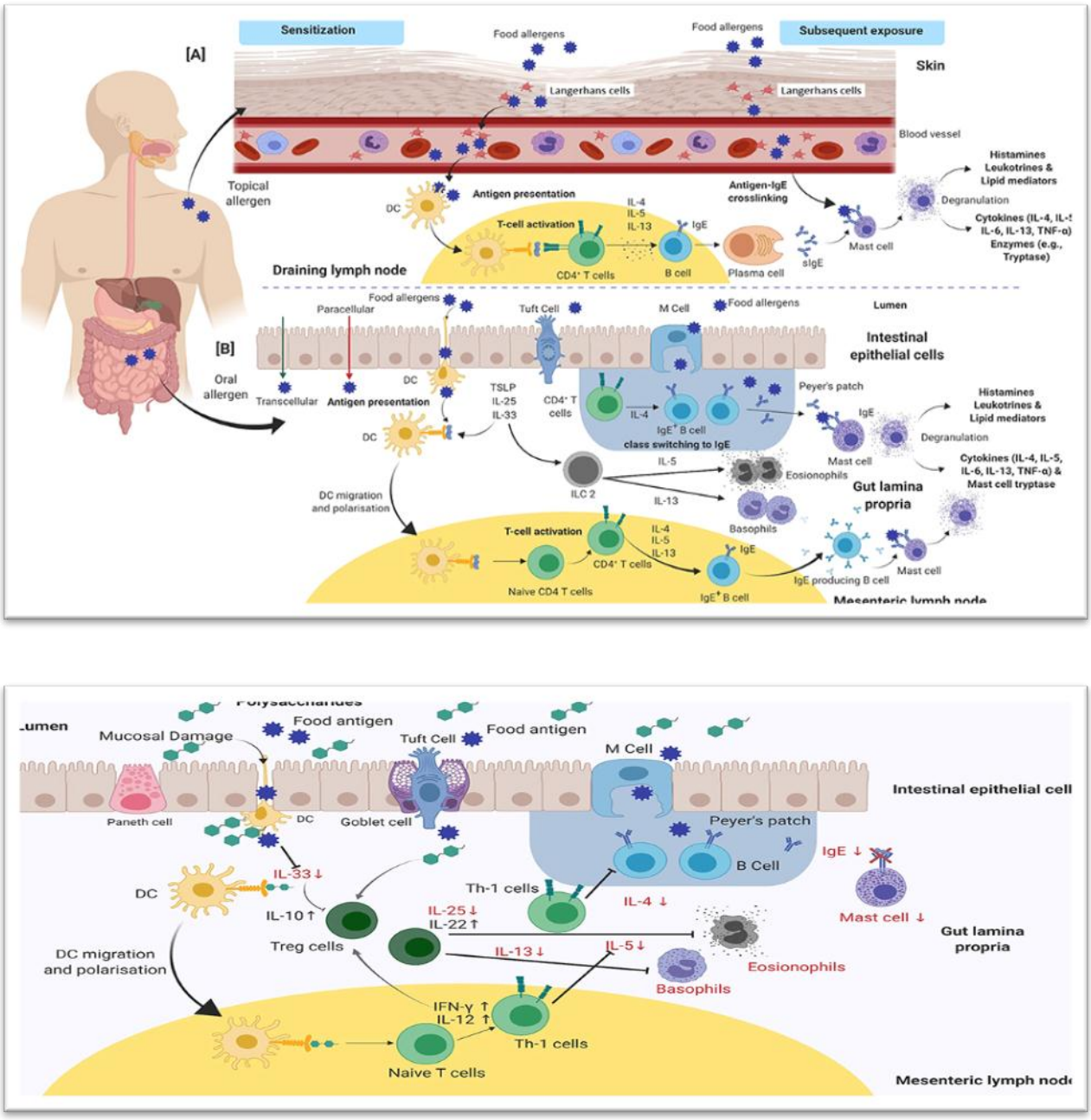

Figure 04.A. An overview of the immunological events occurring during allergic sensitization and effector phase upon exposure to food allergens via. **B.** the effects on different cell populations and cytokines, involved in an allergic immune response, after exposure to polysaccharides, herbal medicinal plants or traditional Chinese medicine (Kunal 2020).

I.9 Conventional treatment

The only "treatment" for food allergies is strict avoidance of allergens. However, new therapies are being developed such as preventive treatment based on the elimination diet and the treatment of anaphylactic shock (Mayorga et al. 2021). The majority of food allergy treatments focus on symptomatic prevention. The first step in treating a food allergy is to implement an allergen

avoidance diet. The goal of this treatment is to eliminate the foods that cause the reactions (Table01) (Long et al. 2020).

Table 01. Main symptoms of food allergy (Soon, Brazier, et Wallace 2020).

Type of reaction	**Target organ**	**Clinical Picture**	**symptomatology**
Skin	Skin	Atopic dermatitis	• Eczema lesions (poorly limited, erythematous) on the face, areas of muscle extension, seat, flexion folds. • Pruritus
	skin	Acute Urticaira	•Eruptive dermatosis due to dermal edema secondary to vasodilation and increased capillary permeability. • Presence of edematous, itchy pink papules.
	mucous	Angioedema or angioedema	•Dermatosis due to hypodermic edema which can be fatal if it affects the pharyngo-laryngeal mucosa. • Pinkish white swelling, not itchy but accompanied by a feeling of tension.
Buccogastrointestinal	Oral mucosa	Oral lessof syndrome	•Labial, gingival, oral pruritus and edema, even edema of the glottis.
	intestine		• Nausea, vomiting, abdominal pain, diarrheal episodes, gastroesophageal reflux.
Respiratory	Nasal mucosa	Rhinoconjonctiva	•Nasal obstruction and itching (inflammation of the mucous membrane), coughing and sneezing attacks, conjunctivitis
	lung	Asthma	• Bronchial constriction leading to respiratory discomfort, with wheezing dyspnea due mainly to histamine release.
systemic		Anaphylactic shock	• Acute circulatory failure, caused by primary peripheral vasodilation linked to the massive release of mediators. • Vital prognosis is at stake.

I.9.1 H1 antihistamines

Antihistamines constitute a major therapeutic class in allergology but are not part of emergency treatments anaphylactic shock, laryngeal edema. They also address itching, runny nose, sneezing and watery eyes. These drugs provide competitive, reversible and specific antagonism of histamine H1 receptors (Chu et al. 2022).

I.9.2. Adrenaline

Despite the elimination diet, accidental ingestion of the allergen can lead to a serious reaction called anaphylaxis. The speed of adrenaline administration has a direct impact on the prognosis. It is based on the rapid administration of adrenaline to prevent the granulation of basophils and mast cells. The intramuscular route is preferable to the subcutaneous route due to a better kinetic profile. The recommended route of administration of adrenaline is intramuscular, in the thigh, using a specialized auto-injector, at a dose of 0.15 to 0.25 mg for children and 0.3 to 1 mg for adults.Hospital care is necessary because vascular filling is essential due to hypovolemia due to anaphylactic shock (Trujillo et Cronin 2022).

I.9.3. Corticosteroids

Corticosteroids are anti-inflammatory medications that may be prescribed to reduce inflammation and persistent symptoms caused by an allergic reaction. They are usually administered as tablets, inhalers, or topical creams, depending on the symptoms and their location (Suryana 2020).

I.10 Alternative treatment for food allergy

Alternative treatments for food allergies encompass unconventional methods and therapies beyond mainstream medical interventions. These approaches often diverge from traditional pharmaceutical treatments and may include modalities such as immunotherapy, herbal remedies, dietary modifications, and mind-body practices. Immunotherapy involves gradual exposure to allergens to desensitize the immune system, while herbal remedies like butterbur and quercetin are believed to possess anti-inflammatory properties that could alleviate allergic reactions (Table02) (Costa et al. 2020).

Table 02. Emergency treatments for adverse reactions linked to the ingestion of a food (Bottinelli et al. 2020).

Severity of reaction	Treatment
Mild (skin reaction only)	Oral antihistamine
Severe (respiratory and/or cardiovascular reaction and/or serious discomfort)	- Adrenaline intramuscularly: 0.1 mg/kg of weight (maximum 0.3 to 0.5). Repeat according to the initial response 5 minutes later. -Antihistamines orally (or intravenously). -Intravenous corticosteroids.

Additionally, hypoallergenic diets and mind-body practices like yoga and meditation aim to mitigate allergy symptoms by promoting overall well-being and immune system resilience. While alternative treatments offer diverse options for managing food allergies, their efficacy and safety require careful evaluation and should be discussed with healthcare professionals to ensure informed decision-making and personalized care (Fiocchi, Vickery, et Wood 2021).

I.10.1 Immunotherapy

Immunotherapy for food allergies involves exposing the immune system to small, controlled amounts of the allergen to desensitize the body's reaction over time (Muraro, Tropeano, et Giovannini 2022). There are different forms of immunotherapy, including oral immunotherapy (OIT) and sublingual immunotherapy (SLIT). OIT involves ingesting small, gradually increasing doses of the allergen, while SLIT involves placing drops of allergen extract under the tongue. While research into immunotherapy for food allergies is promising, it's still considered experimental and should only be conducted under the guidance of an allergist or immunologist (Schoos et al. 2020).

I.10.2 Phytotherapy

Phytotherapy, also known as herbal medicine, involves using plant extracts and natural substances to treat various conditions, including allergies. Some herbs and plant extracts are believed to have anti-inflammatory or antihistamine properties, which may help alleviate allergy symptoms (Zhang et al. 2022). Examples include butterbur, quercetin, and stinging nettle. However, scientific evidence supporting the effectiveness of phytotherapy for food allergies is limited, and the safety and efficacy of these treatments can vary widely (Kumphanda, Mtewa, et Baluwa 2021).

Medicinal plants are a rich source of bioactive compounds that offer numerous health benefits. These compounds include alkaloids, flavonoids, terpenoids, glycosides, and polyphenols, among others. For instance, alkaloids (Butranui 2022).

Terpenoids, such as menthol from peppermint, provide soothing effects and are widely used in respiratory therapies (Singh 2015). Polyphenols, abundant in green tea, have been shown to reduce the risk of cardiovascular diseases and cancer and food allergy and microbiota modulation due to their strong antioxidant properties. The therapeutic potential of these bioactive compounds underpins the importance of medicinal plants in traditional and modern medicine, offering natural

remedies for a range of ailments and contributing to the development of new pharmaceuticals (Mancini 2017).

- **Dietary Polyphenols and Their and their impact on food allergy**

Dietary polyphenols, present in many foods, have emerged as promising candidates for developing new therapeutic approaches to both prevent food allergies and alleviate associated symptoms. With over 8000 phenolic compounds synthesized by plants, these compounds exhibit significant structural diversity. As products of the secondary metabolism of plants, phenolic compounds are found in all plant-based foods and their derivatives (Figure05) (Table03) (Simões, 2024).

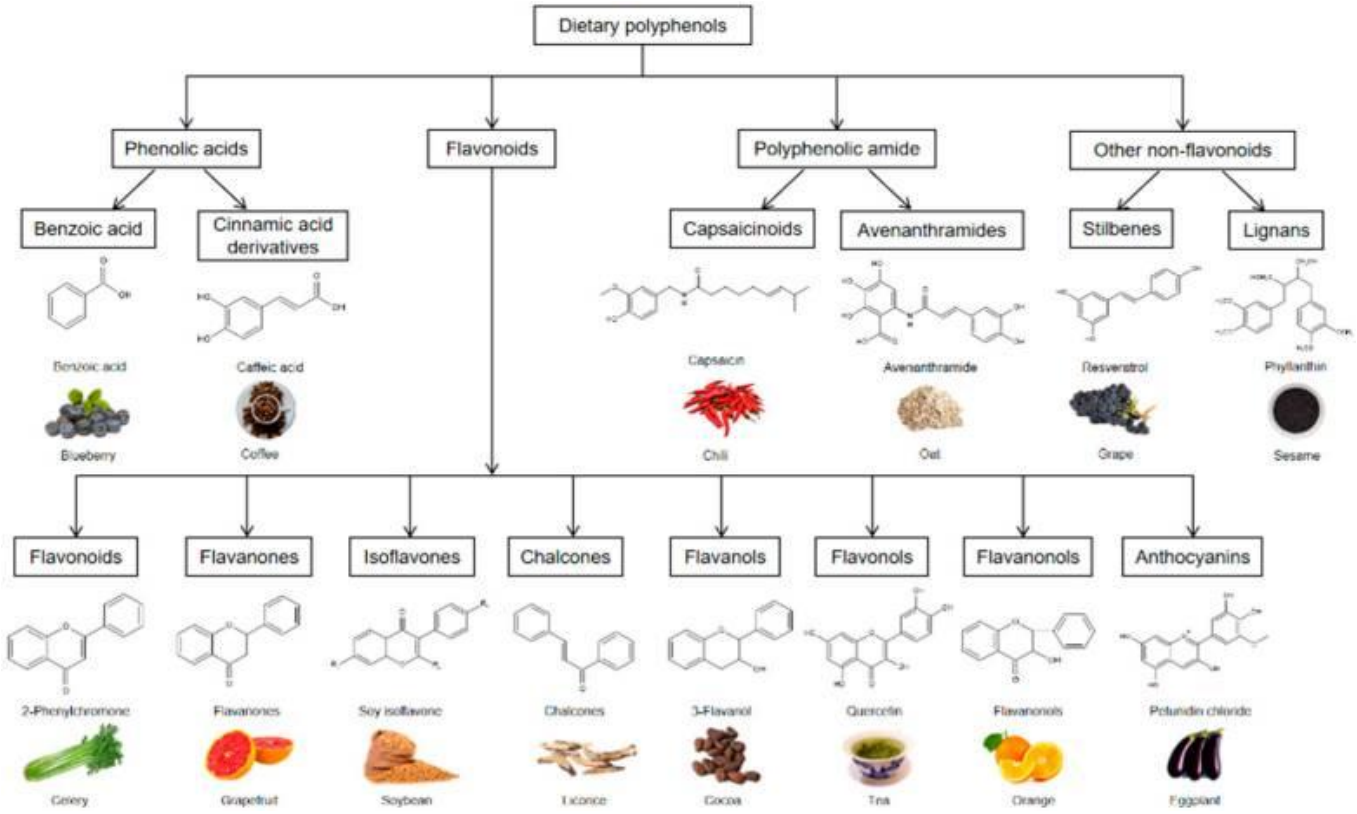

Figure 05. Classification of dietary polyphenols and their sources.(Wang, Qi, et Zheng 2022).

Table 03. Phenolic compounds can be used as a powerful tool in controlling the prevalence of food allergies. (Simões, 2024).

Phenolic compound	Experimental study	Biological action	Food allergy
Epigallocatechin gallate	Protein–phenolic compounds complexation	Conformational changes	Milk allergy (albumin)
Epigallocatechin gallate	Protein–phenolic compounds complexation	Conformational changes	Shrimp allergy (tropomyosin)
Resveratrol	Mouse model	Inhibition of Th2 differentiation and antigen presenting cells (APCs)	Ovalbumin
Red wine and coffee phenolic compounds	In vivo gut microbiota	Increase Bacteroides	Inflammation biomarkers of allergic rhinitis
Apple Phenolic compounds extract	Mouse model	Reduction of allergy symptoms in adose-dependent manner	Ovalbumin
Apple phenolic compounds extract	In vitro mast cell degranulation	Reduced histamine release	Universal allergy model
Phenolic acids	Protein–phenolic compounds complexation	Binding to peanut allergy-specific IgE	Peanut allergy

I.11 Prevention

There are limited data on primary prevention of food allergy through dietary means, although numerous studies possessing various limitations have addressed outcomes of atopic disease, such as atopic dermatitis and asthma. Based on review of the available literature, professional organizations have generally concluded that there is insufficient evidence regarding reduced atopic disease to recommend maternal avoidance of allergens during pregnancy or lactation, although there is some (Sicherer et Sampson 2009).

II. Food Allergy and dysbiosis

II.1 Microbiota

The intestinal micro biota is a complex ecosystem that includes all of the unicellular beings housed in the digestive tract, mainly bacteria but also viruses, fungi and archaea. After colonization of the digestive tract from birth to the age of approximately 2 years, the intestinal micro biota is specific to each individual and stable in the time (Liang et al. 2023). Furthermore,

there is a phenomenon of resilience, that is to say the return to balance after a disruptive event (for example taking antibiotics) (Carding 2015).

II.2. Dysbiosis and microbiota

Dysbiosis refers to an imbalance in the microbial community in the body, particularly in the gut. This imbalance can involve an overgrowth of harmful bacteria, a decrease in beneficial bacteria, or a shift in the overall composition of the micro biota (Carding 2015). The main causes of dysbiosis are viral, bacterial or parasitic infections, sudden changes in diet, immune deficiencies and use of medications that can modify the intestinal flora (Sicherer et Sampson 2009).

Dysbiosis in the gut microbiota has been associated with various health conditions, including inflammatory bowel diseases, obesity, and metabolic disorders. Maintaining a healthy balance of microbiota through diet, lifestyle, and sometimes medical interventions is crucial for overall health and well-being (Carding 2015).

II.3. Food allergy and microbiota

The intricate relationship between food allergies and the microbiota underscores the pivotal role that gut microorganisms play in immune regulation and overall health. Dysbiosis, has been shown to be implicated in the development of food allergies. Research suggests that a diverse and balanced microbiota early in life may foster immune tolerance, thereby reducing the risk of allergic reactions to food antigens (Ali, Tan, et Kaiko 2020).

Moreover, the microbiota contributes to the integrity of the gut barrier, preventing the passage of food antigens into the bloodstream and mitigating allergic responses. Short-chain fatty acids (SCFAs) produced by beneficial gut bacteria further modulate immune function, potentially diminishing the likelihood of food allergy development.

Therapeutic strategies are currently aiming to modulate the microbiota, such as probiotics, prebiotics, and fecal microbiota transplantation (FMT), hold promise for mitigating food allergy risks. Continued research into the complex interplay between food allergies and the microbiota offers insights into preventive and therapeutic interventions to promote gut health and alleviate allergic manifestations (Figure06) (Carding 2015).

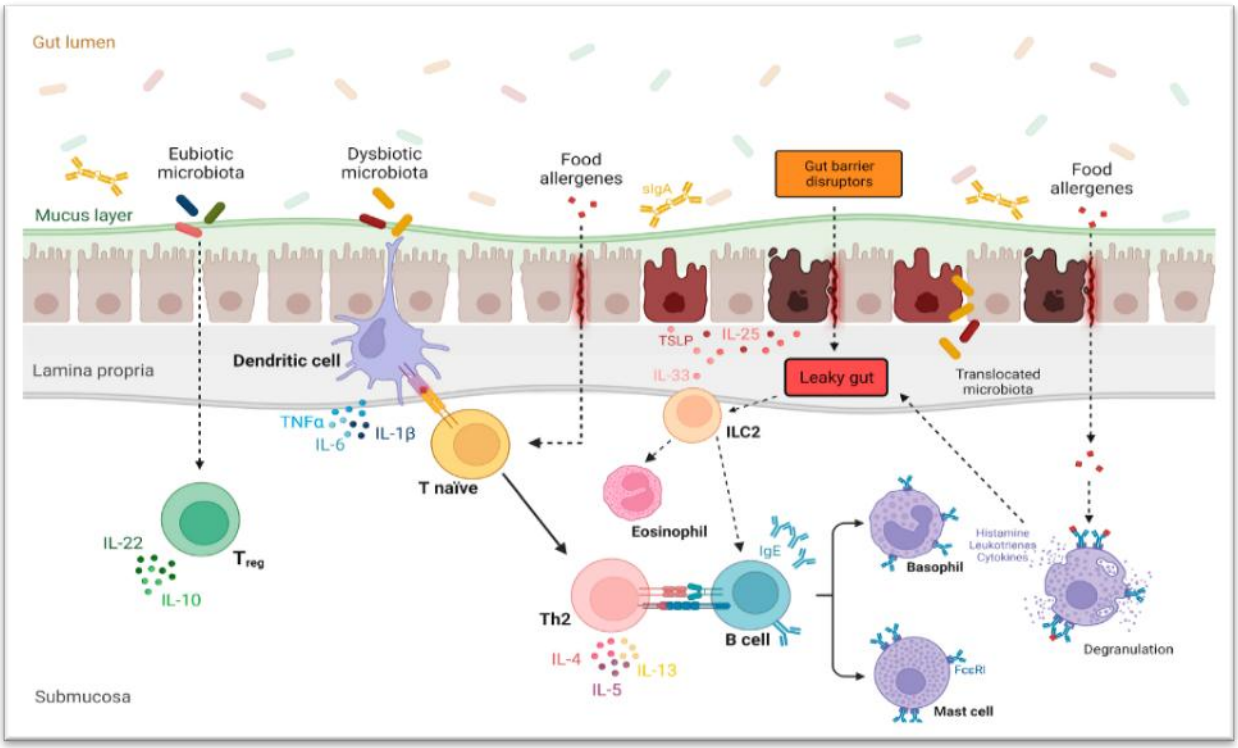

Figure 06. The role of gut microbiota and leaky gut in the pathogenesis of food allergy (Simões, 2024).

Under physiological conditions, a healthy gut microbiota (eubiotic) encourages the differentiation of T lymphocytes into T regulatory (Treg) cells, fostering immune tolerance within the gut. However, in situations involving a leaky gut barrier and intestinal dysbiosis, epithelial-derived cytokines like thymic stromal lymphopoietin (TSLP), interleukin 33 (IL-33), and IL-25 are released in response to various gut barrier disruptors. These cytokines, known as alarmins, create a pro-allergic environment by activating T helper 2 (Th2) and type 2 innate lymphoid cells (ILC2), which then release proinflammatory cytokines such as IL-4, IL-5, and IL-13, Moreover, dysbiotic gut microbiota stimulate the differentiation of Th2 cells, which supports the IgE class-switching process in B cells.

This class-switching process results in the production of allergen-specific IgE antibodies, which attach to the surface of basophils and mast cells. Upon re-exposure to the allergen, these cells release histamine and other proinflammatory mediators, including leukotrienes and type 2 cytokines. This release exacerbates gut permeability and amplifies type 2 inflammation, perpetuating the cycle of allergic responses and gut barrier dysfunction. Abbreviations:FcεRI: High-affinity IgE receptor,IL: Interleukin,IgE: Immunoglobulin E,ILC2: Innate lymphoid cell 2,Th0: Naive T cell, Th2: Helper T cell 2, Treg: T regulatory cell,TLSP: Thymic stromal lymphopoietin,sIgA: Secretory IgA. (Poto et al. 2024).

II.4 Food allergy and oxidative stress

Oxidative stress refers to an imbalance between the productions of reactive oxygen species (ROS) and the ability of the body to detoxify or neutralize their harmful effects through antioxidants. ROS, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are natural byproducts of normal cellular metabolism and play essential roles in various physiological processes (Choo et al. 2021).

The relationship between oxidative stress and allergic sensitization is intricate due to a dual role played by allergen-induced oxidative stress. On one hand, allergen-induced oxidative stress can initiate the Th2 immune response, contributing to allergic sensitization. Conversely, the inflammatory process triggered by the immune response generates endogenous oxidative stress. Thus, while allergen-induced oxidative stress can initiate the allergic response, inflammation itself also leads to the production of oxidative stress within the body. This dynamic interplay underscores the complexity of the relationship between oxidative stress and allergic sensitization (van Rijt et al. 2017).

Conversely, in hosts with sufficient antioxidant responses, allergen-induced oxidative stress alone may not induce allergic sensitization unless accompanied by a stronger stimulus, such as the activation of Toll-like receptor 4 (TLR4). This highlights the role of antioxidant defenses and additional signals in modulating the outcome of allergen exposure and the development of allergic responses. (van Rijt et al. 2017).

Chapter two

Phytotherapy and studied plants

I. Medicinal plants

Definition

Medicinal plants are used because therapeutic properties for human health. In fact, they are used in different ways, decoction, infusion and maceration. One or more parts of them, roots, leaves, flowers can be used (Shikov et al. 2021). These multiple therapeutic effects that come from their compounds (primary metabolites or secondary) or the synergy between the different existing compounds (Salmerón-Manzano, Garrido-Cardenas, et Manzano-Agugliaro 2020). According to the WHO, more than 20,000 plants are used worldwide in folk medicine, but only 2000 to 3000 plants have been studied at the scientific level (Li et al. 2020).

I.1. Medicinal plants and food allergy

Medicinal plants have long been explored for their potential in alleviating allergy symptoms. Many plants contain compounds with anti-inflammatory, antihistaminic, and immunomodulatory properties, which can help mitigate allergic reactions (Pratap et al. 2020). For example, nettle leaf extract is known for its anti-inflammatory effects, while turmeric contains curcumin, a compound studied for its ability to reduce inflammation. Ginger, another medicinal plant, contains gingerol, which has demonstrated anti-inflammatory properties.

Additionally, compounds like quercetin, found in onions and apples, and butterbur extract have been investigated for their potential to stabilize mast cells and reduce the release of histamine, thus offering relief from allergies. However, it's crucial to approach the use of medicinal plants cautiously and under the guidance of a healthcare professional, as they may cause adverse effects or interact with medications (Rahim et al. 2021).

I.2. Studied plants

Figure 07. *Rosa damascene*

I.2.1. Rosa damascena

Rosa damascena (RD) (Table 3), is a pink rose that is a cultivated hybrid, this plant is cultivated in Iran, It is one of the most important species of Rosaceae family, is utilized for its therapeutic properties in traditional medicine and food industry, *R.damascena* is mainly known for its perfuming effects. *Rosa damascena* was frequently used as astringents for the treatment of gastric disorders and inflammation of gastric mucous membranes (Figure 07)(Table04) (Boskabady et al. 2011).

- **Therapeutic properties**

Different pharmacological effects of *R.damascena*, have shown multiple positive benefits, its The analgesic effect, antitussive effects , Hypnotic effect, Neuropharmacolgical effects, Anti-diabetic, Antioxidant, Bronchodilatory, Anticonvulsant, Antibacteria, Laxative and prokinetic Anti-inflammatory (Bożena , 2016). *R.damascena,* shows great potential for a traditional solution in today's world as a preservative and more eminently a therapeutic agent.

Table 4. Classification of *Rosa damascene* (Bożena , 2016)

kingdom	**Plantae**
phylum	Magnoliophyta
class	Rosidae
Order	Rosales
Family	Rosaceae
Genus	Rosa
Species	*Rosa damascena*

I.2.3. *Origanum majorana*

Origanum majorana.L, is an aromatic and medicinal, this plant distributed round the Mediterranean regions, This species is widely used in traditional medicine for the treatment of many diseases such as allergies, hypertension,respiratory infections, diabetes, stomach pain, and intestinal antispasmodic. (Figure8)(Table5) (Bouyahya et al. 2021).

- **Therapeutic properties**

The phytoconstituents of *Origanum majorana. L* have Show antibacterial, calming effects,

anti-inflammatory and antispasmodic properties, making it valuable in alleviating muscle spasms (cramps), and digestive issues like bloating and indigestion *(*Algebaly, 2021).

Figure 08. *Origanum majorana.L (*Algebaly, 2021).

Table 5. Classification of *Origanum majorana. L* (FIGUEREDO, 2007).

kingdom	**Plantae**
phylum	Spermaphyte
class	Dicotyledons
Order	Lamiales.
Family	Lamiaceae
Genus	Origanum.
Species	majorana L.

I.3. Main secondary metabolites from the studied plants and their impact on bacterial growth:

The chemical composition of *O.majorana* and *R.damascena.*, of secondary metabolites (terpenoids and polyphenols in our case), consists of numerous bioactive compounds that are responsible for its gourmet and therapeutic qualities. The table below offers a comprehensive overview of the major secondary metabolites identified in (Table 06).

Table 6. Main secondary metabolites characterized in O.majorana.

Component	**Concentration**	**Properties**	**Referances**
Essential oils		Consisting of terpene or sesquiterpene hydrocarbons,	
Terpinene-4-ol	19-26%	Antimicrobial, anti-inflammatory	(Sienkiewicz et al. 2011)
γ-Terpinene	11-15%	Antioxidant, antimicrobial	(Güllüce et al. 2003)
α-Terpineol	5-7%	Antiseptic, antioxidant, sedative	(Peana et al. 2002)
Sabinene Hydrate	3-6%	Antimicrobial	(Güllüce et al. 2003)
Linalool	3-10%	Antibacterial, antifungal, pleasant aroma	(Silva et al. 2003)
Phenolic compounds		Hight in antioxidants and contribute to therapeutic properties	
Rosmarinic Acid	1.5-3%	Strong antioxidant, anti-inflammatory, and antimicrobial properties.	(Petersen et Simmonds 2003)
Caffeic Acid	0.5-1.2%	Antioxidant, anti-inflammatory, and anticarcinogenic effects.	(Son et Lewis 2002)
Luteolin	0.1-0.5%	Anti-inflammatory, antioxidant, and neuroprotective effects.	(Petersen et Simmonds 2003)
Apigenin	0.1-0.5%	Antioxidant, anti-inflammatory, and anticancer properties.	(Shukla et Gupta 2010)
Thymol	0.2-0.8%	Strong antimicrobial and antioxidant properties	(Sienkiewicz et al. 2011)
luteolin, quercetin		Anti-inflammatory, antioxidant, neuroprotective effects., and anticancer properties.	(Sellami., et al, 2012).

Table 7. Main secondary metabolites of R.damascena.

Component	Concentration	Properties	Referances
Essential oils		Consisting of terpene or sesquiterpene hydrocarbons,	
Citronellol	30-40%	Citronellol is known for its strong antimicrobial, anti-inflammatory, and insect-repellent properties. It also contributes to the pleasant floral scent of the oil.	(Verma, 2011).
Geraniol	10-20%	Geraniol has antimicrobial, antioxidant, and anti-inflammatory properties. It is also used in aromatherapy for its calming effects.	
Nerol	5-10%	Nerol is known for its pleasant aroma and its antimicrobial and anti-inflammatory activities. It is also used for its calming effects in aromatherapy.	(Weiss, 1997).
Phenyl Ethyl Alcohol	2-3%	This compound has a sweet, floral fragrance and exhibits antimicrobial properties. It is often used in perfumery and cosmetics.	(Arctander, 1960).
Nonadecane	8-15%	Nonadecane contributes to the fixative properties of the oil, helping to stabilize the fragrance. It also has antimicrobial properties.	(Hajian, 2010)
Eugenol	0.5-1.5%	Eugenol is known for its strong antimicrobial and analgesic properties. It also contributes to the spiciness in the fragrance profile of the oil.	(Shabir, 2009).
Phenolic compounds	Variable	These compounds contribute to its antioxidant, anti-inflammatory, and antimicrobial activities.	
Quercetin	Variable	Quercetin exhibits strong antioxidant and anti-inflammatory properties. It helps in scavenging free radicals and reducing inflammation.	(D'Andrea 2015)
Kaempferol	Variable	Kaempferol has antioxidant, anti-inflammatory, and anticancer properties. It helps in neutralizing free radicals and reducing inflammation.	(Calderón-Montaño, 2011).
Rutin (Quercetin-3-O-rutinoside)	Variable	Rutin is known for its antioxidant and anti-inflammatory properties. It helps in scavenging free radicals and reducing inflammation.	
Catechin	Variable	Catechin exhibits antioxidant and anti-inflammatory properties. It helps in neutralizing free radicals and reducing inflammation.	(Higdon, 2003).
Kaempferol, quercetin, rutin		Anti-inflammatory, antioxidant, neuroprotective effects., and anticancer properties.	(Sellami, 2012)

Table 8. Modulatory effect of O.majorana on the growth of some bacterial strains.

Category	**Details**	**Reference**
Enriched Bacteria		
Lactobacillus spp.	Beneficial bacteria that may be promoted by the phenolic and flavonoid compounds in Origanum majorana, contributing to gut health and immune function.	(Beghelli, D., 2014)
- *Bifidobacterium spp.*	Similar to Lactobacillus, these bacteria are beneficial for gut health and may be enriched by the prebiotic effects of Origanum majorana compounds.	(Beghelli, D., 2014)
Depleted Bacteria		
E.coli	Pathogenic bacteria inhibited by the antibacterial compounds in Origanum majorana, such as carvacrol and thymol.	(Nostro, A., et al. 2004).
S.aureus	Another pathogenic bacterium inhibited by the antibacterial action of Origanum majorana's essential oils.	(Nostro, A., et al. 2004).
Pseudomonas aeruginosa	Pathogenic bacterium affected by the antimicrobial properties of the phenolic and terpenoid compounds in Origanum majorana.	(Nostro, A., et al. 2004).

Table 9. Modulatory effect of R.damascena on the growth of some bacterial strains.

Category	**Details**	**Reference**
Enriched Bacteria		
Lactobacillus spp.	Beneficial bacteria that may be promoted by the prebiotic effects of phenolic and flavonoid compounds in Rosa damascena.	(Nostro, A., et al. 2004).
- *Bifidobacterium spp.*	Similar to Lactobacillus, these bacteria are beneficial for gut health and may be enriched by the prebiotic effects of Rosa damascena compounds.	(Nostro, A., et al. 2004).
Depleted Bacteria		
E. coli	Pathogenic bacteria inhibited by the antibacterial compounds in Rosa damascena, such as gallic acid and citronellol.	(Basim, 2003)
S.aureus	Another pathogenic bacterium inhibited by the antibacterial action of Rosa damascena's essential oils.	(Basim, 2003)
Pseudomonas aeruginosa	Pathogenic bacterium affected by the antimicrobial properties of the phenolic and terpenoid compounds in Rosa damascena.	(Basim, 2003)

Materials and Methods

Our research work was carried out between February and April 2024, at the Unité de Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques, Université des Frères Mentouri Constantine, and the Applied Microbiology Laboratory, Faculty of Nature and Life Sciences, Université Des Frères Mentouri Constantine.

I. Materials

I.1. Plants' material

Air dried aerial parts of *Origanum majorana.L,* and *Rosa damascena,* were purchased from a local market from the Wilaya of M'sila, Algeria in February 2024. These plants are sold as medicinal plants for folk medicine treatment, as cosmetic ingredients, and as culinary additives. Both plants were identified by Dr. RAMLI, from the Department of Biochemistry and molecular and cellular Biology, Université des Frères Mentouri Constantine1. Plants' material was grounded into a fine powder using an electric mill (Moulinex), then stored at room temperature (25°C) until use.

I.1. Bacterial strains

Two pure pathogenic bacterial strains, namely *Staphylococcus aureus* and *Escherichia coli*, obtained from the ATCC (Aida et al. 2022). These strains were supplied by the Microbiology Laboratory. and They are represented in the following (table 10).

Table 10. The tested bacterial strains (Aida et al. 2022).

species	N°ATCC	Gram	Family
E. coli	NCTC10.538	Gram negative	Enterobacteriaceae
S. aureus	ATCC 6538	Gram positive	Staphylococcaceae

II. Methods

II.1. Extraction by ultrasonication

- The extracts were obtained by performing ultra-sonification method in absolute methanol (Cheriet et al. 2017). A total weight of 102,46 g and 99,56 g of *R.Damascena* and *O.majorana* respectively was macerated at room temperature (25°C) in absolute methanol MetOH for 1 hour using an ultrasound (Fisherbrand). the macerate was then filtered and condensed using a rotative evaporator (BUCHI) and were then condensed and conserved at

- room temperature until next use. The extracts were referred to as RD for the extract of *R.damascena*, and as OM for the extract of *O.majorana*. The extraction ratio was calculated using the following equation:

$$yield\ of\ extraction(\%) = \frac{the\ wieght\ of\ extract}{initial\ weight\ of\ plant\ powder}\ x100$$

These extracts were used in the following tests: Total phenols content, antioxidant activity using DPPH, total flavonoids content, Antibacterial activity.

II .2. Chemical screening

Reagents

Unless stated in the text all chemicals reagents were purchased from euroclone.

II .3. Total phenolic content(TPC)

The total phenolic content refers to the concentration of all phenolic compounds present in a sample(Mircea et al. 2015).TPC is often measured spectrophotometrically using the Folin-Ciocalteu assay, where phenolic compounds react with Folin-Ciocalteu reagent and sodium carbonate to produce a blue coloration, which is then quantified based on the optic absorbance (Fadda *et al*., 2016; Singleton and Rossi, 1965).

The TPC is expressed as milligrams of gallic acid equivalents (GAE) per gram of sample(Ikram et al. 2009). The TPC was evaluated following the method performed by Briefly, The extract was prepared with a concentration of 1mg/ml in distilled water ,then 250 µl a methanolic solution of extracts (1 mg/ml) were mixed with 500 µl of Folin-Ciocalteu reagent (1N) and left to react for 4min incubation at 25° C in the dark ,then 250 µL sodium carbonate solution (Na2CO3)(20%) was added before incubating in the dark for 120 min at room temperature (25 °C). The optical absorbance was read at 760 nm with a spectrophotometer (therma), the experiments were repeated in triplicate and results were expressed as milligrams of Gallic acid equivalent/g of dry weight on the basis of a Gallic acid calibration curve. The Gallic Acid solution was prepared under the same conditions at an initial concentration of 1mg/mL in water (Ikram et al. 2009).

II.4. Total flavonoids content (TFC)

Flavonoids content in a sample can be determined spectrophotometrically using the aluminum chloride colorimetric assay. In this assay, flavonoids react with aluminum chloride to form a complex that absorbs light at specific wavelengths, allowing their quantification. Flavonoid content is typically expressed as milligrams of a specific flavonoid equivalent (e.g., quercetin or rutin) per gram of sample (Kalita et al. 2013). Briefly, The extract was prepared with a concentration of 1mg/ml in methanol, then 1ml of each extract was mixed with 1ml of aluminum trichloride solution (ALCL3 2%), and the absorbance was measured at 420 nm after 1hour of incubation, the experiment was performed in triplicate. How did you express the content (Topçu *et al*., 2007).

II.5.Antioxidant activity using DPPH

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical commonly used to evaluate the antioxidant activity of compounds. This method quantifies the ability of antioxidants to donate hydrogen atoms or electrons to neutralize free radicals, thus indicating their potential to protect cells from oxidative (Kalita et al. 2013). the reference of the method. Briefly, A stock solution of extract was prepared in methanol with a final concentration of 8mg/mL. Then a series of ½ dilutions was prepared ranging from 8mg/mL to 0,125mg/mL (Molyneux 2004).

A methanol solution of DPPH was prepared at a final concentration of 0,04 mg/mL. Then a volume of 50 µL of the extract solution is added to 3 mL of DPPH solution. the experiment was carried out in triplicate. A negative control is prepared by mixing 50 µL of methanol with 3ml of the methanolic DPPH solution then the absorbance was measured at 517 nm after 30 min of incubation in the dark at room temperature. Ascorbic acid was used as a standard and was prepared under the same experimental conditions with the concentration of 1mg/L. The percentage of DPPH inhibition for each concentration was calculated using the following equation:

$$DPPH(\%) = \frac{(A\ control - A\ extract)}{A\ control}\ x100$$

II.6. Antibacterial activity

The antibacterial activity assay of the obtained extracts was evaluated by wells diffusion (Hsouna et al., 2011) slightly modified using Gram positive (*Staphylococcus aureus (ATCC 6538))*, and Gram negative species (*Escherichia coli* NCTC10.538)). Microorganism inoculums were standardized to a turbidity equivalent to 0.5 McFarland standards (108 UFC/ml bacterial cells or 107 yeasts)., the microbial inoculum spread over the surface on the surface of the medium, then, using a sterile rod, a well with a diameter of 6nm was created aseptically. Finally, 60 µL of the tested antimicrobial agent (plant extract 100 mg/mL) was filled into the well. The inhibition diameter was measured after 24h of incubation at 37 °C.

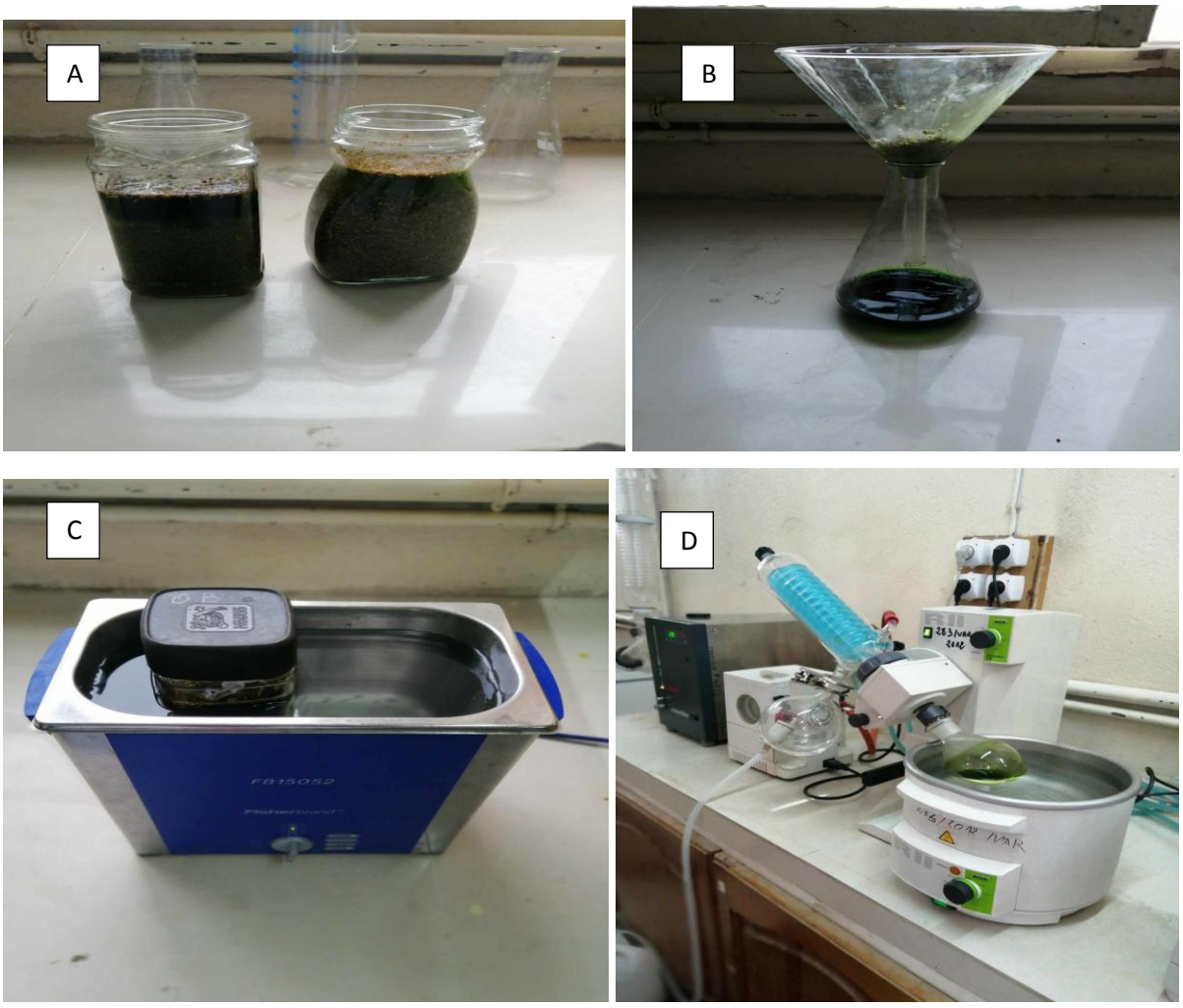

Figure 9. Extraction by ultrasonication of OM. A: Maceration of plants, B: filtration, C: the plants in ultrasound, D: the plants in a rotative evaporator.

Figure 10. Dilutions Antioxidant activity using DPPH

Figure 11. Zone of inhibition of OM and RD.

II.7. Statistical analysis

Data for all tests were expressed as mean ± SD of measurements repeated in triplicate. One-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test were performed in DPPH experiment to determine means of differences between treatments with a P value significance at (P < 0.05). Statistical analysis was performed using GraphPad Prism version 9.00 (GraphPad Software, San Diego California USA).

Results and Discussion

1. Extraction yields

The obtained quantities (g) and yield of each extract (%) are shown in table 10. Our plants *O. majorana* and *R.damascena* obtained a yield of extraction of 3,87%and 10,21%respectively (table 11) for the ultrasonification method.

Table 11. yield of *O. majorana. and R.damascena*

Extract	**Weight (g)**	**quantities of extract (g)**	**Extraction yield (%)**
OM	99,56 g	3,853 g	3,87%
RD	102,46g	10,47g	10,21%

OM: *extract Origanum majorana.L RD :extract of Rosa damascene*

2. Total phenolic content

An important phenolic content was shown by our extracts, with OM showing the highest amount of 660,33±35,37 (µg QE/mg), while the RD extract recorded a value of 639,80±106,43 (µg QE/mg) (Table 12).

Table 12. total phenols content of *O. majorana.L and R.damascena.*

Plants	**Total phenols content (µg GAE/g±SD)**
O. majorana	660,33±35,37
R.damascena	639,80±106,43

OM: *extract of Origanum majorana.L RD :extract of Rosa damascene;* (µg QE/mg: µg of quercetin equivalent equivalent /g of dry plant extract weight ;SD: Standard of deviation. /: non-defined. Values are expressed as means ± SD of three parallel measurements.

3. Total flavonoids content

The assessment of total flavonoids revealed that *R.damascena* showed the highest flavonoid content, with a substantial amount of 93,50 ± 8,97 µg QE/mg. In contrast, the *O. majorana.* extract exhibited a mean value of 85,12±11,03µg QE/mg (Table13).

Table 13. Total flavonoids content of *O. majorana.L and R.damascena.*

plants	**Extract**	**Total flavonoids content (µg QE/mg±SD)**
O. majorana.L	OM	85,12±11,03
R.damascena.	RD	93,50±8,97

OM:extract of *Origanum majorana.L RD:* extract of *Rosa damascene*; (µg QE/mg: µg of gallic acid equivalent /mg of dry plant extract weight SD: Standard of deviation. /: non-defined. Values are expressed as means ± SD of three parallel measurements.

4. Antioxidant activity using DPPH

Both extracts demonstrated significant antioxidant potency where the RD extract showed the highest IC_{50} value of 4,57±0,03 µg/Ml represents two folds the standard value p $p < 0.05$, while the OM extract also exhibited a low IC_{50} value at 2,54 ±0,01 which was slightly different from the IC_{50} of ascorbic acid at 2,95±0,25 µg/ Ml $p < 0.05$ (Table14).

Table 14. Antioxidant activity of *O.majorana.L and R.damascena*

Samples	**IC_{50} DPPH (µg /mL±SD)**
OM	$2,54 \pm 0,01^{a}$
RD	$4,57 \pm 0,03^{b}$
Ascorbic acid	$2,95 \pm 0,25^{c}$

OM: *extract of Origanum majorana* . RD: extract of *Rosa damascene* ;SD: Standard deviation ; IC50:sample concentration at which 50% of the free radicals activity is inhibited; DPPH: radical scavenging assay. Values are expressed as means ± SD of three parallel

measurements. Values that do not share the same letters are significantly different ($p < 0.05$ for one-way ANOVA and Tukey multiple comparison tests).

5. Antibacterial activity

Our extracts were evaluated against two bacterial strains using the well diffusion method as described slightly modified. The RD extract produced an 8 mm zone of inhibition against *S. Aureus* and 10mm against E. Coli. While OM extract demonstrated strong antibacterial activity against both bacterial strains, with inhibition zones of 20 mm against *E.Coli* and 7 mm against S. Aureus. These findings indicate that OM and RD Ancompasses a more extensive range of antibacterial activity (Table 15).

Table 15. Antibacterial activity of *O. majorana.L and R.damascena.*

Bacterial strain	**Plant extract**	
	OM	RD
E.Coli	20mm	10mm
S. aureus	7mm	8mm

OM: *Origanum majorana.L RD : Rosa damascena*

Discussion

Emerging evidence links the rising prevalence of food allergies to changes in the composition and function of gut microbiota. As a result, probiotics have garnered significant attention as a preventive and therapeutic approach (Shang, 2018).

O. majorana. is an important genus in Lamiaceae family and is used as herbal tea, flavoring agents and medicinal plant (Stahl-Biskup et Saez 2002). This plant is rich in phytochemicals, including thymol, carvacrol, tannins, hydroquinone, arbutin, methyl arbutin, vitexin, orientin, thymonin, triacontan, sitosterol, cis-sabinene hydrate, limonene, terpinene, camphene, and flavonoids like diosmetin, luteolin, and apigenin, which account for its biological propertie(Stahl-Biskup et Saez 2002).

R. damascena it is one of the most important species of Rosaceae family This plant contains several components such as terpenes, glycosides, flavonoids, and anthocyanins that have beneficial effects on human health. *Rosa damascena* contains carboxylic acid, myrcene, vitamin C, kaempferol and quarcetin. Flowers also contain a bitter principle, fatty oil and organic acids which also been used for medicinal purposes.

Extraction is the initial step in isolating desired natural products from raw materials procedure. The extraction of medicinal plants involves separating active compounds, such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides, from inert or inactive materials. This process uses an appropriate solvent and a standardized extraction procedure (Abubakar, 2020). In this study,We used the extraction assisted by ultrasound method (UAE).

The mechanical effect of ultrasound enhances solvent penetration into cellular materials and disrupts biological cell walls, facilitating the release of their contents. The application of sonochemistry in food processing offers several advantages, including increased extraction yield and rate, reduced extraction time, and higher throughput (Alice, 2012). Methanolic extracts were obtained with a yield of extraction of 3,87% and 10,21% respectively for *O. majorana and R.damascena.* The yield of the *O. majorana* (3,87%) extraction in our study was in the same range obtained by benchikha et al.(2013), Asma Algebaly et al. (2021) and Ezgi Aytaç et al. (2020), who indicated the yields of 8,16%, 14,3%, 4,2% successively from the aerial parts of the same species (Benchikha, Menaceur, et Barhi 2013),(Algebaly et al. 2021), (Aytaç 2020)

The yield obtained by the second plant *R.damascena* 10,21% in the present study was shown to be much lower than the amounts recorded by the study of Mounia Chroho et al. (2022) (40,5%) on the same species (Mounia, 2022).

The extraction yield variation maybe essentially be due to the plant source (Isabel, 2022), extractionmethod (Benchikha, Menaceur, et Barhi 2013)genetic variability (Isabel, 2022). Globally, both phenolic and flavonoid profiles of our extracts revealed considerable levels.

For OM, our findings are in a disagreement with Asma Algebaly et al. (2021), who recorded a value of 58.24 ± 1.82 (mg equivalent/g), which is higher than the registered levels in our study (660,33±35,37 µg GAE/mg), while the total phenols content obtained from the methanolic extract of RD 639,80±106,43 µg GAE/g was also shown to be higher than the values reported by (Mounia Chroho et al. (2022), where they indicated a content of approximately 0.20 ± 0.1 mg/mL (Mounia, 2022).

The concentration of flavonoids was found to be 85,12±11,03 (µg QE/mg) of dry plant for OM, which was very similar to the value reported by (Algebaly et al. 2021), who demonstrated a value of 93,50±8,97 (µg QE/mg), whereas our findings about RD are in concordance with the works of who detected important amounts of total flavonoids in *R.damascena* (0.987 ± 0.05 mg QE/g), along with other varieties of(Mounia, 2022) the same plant.

According to the results of the methanol extract of OM and RD, we may suggest that our extracts are poor in flavonoids and rich terms of polyphenols. Globally phenolic compounds are known to be abundant in all Lamiaceae species which is in line with our results.

The antioxidant activity of our extracts was evaluated using a methanol solution of DPPH and was compared to ascorbic acid. According to our results the studied extracts revealed a strong antioxidant ability with IC_{50} values of 2,54 ±0,01 µg /mL and 4,57±0,03 µg /mL, for *O.majorana*, and *R.damascena* respectively. Different results have been shown a in benchikha et al. (2013) works showing that some fraction extracted from OM demonstrated a mild antioxidant effect compared to our extracts (Mounia, 2022). Besides, the IC_{50} of the RD extract showed a value of 4,57±0,03 µg /mL which is different from results that have been shown in (Antoaneta, 2023).

Our extract possesses an important antioxidant efficiency. which can replace synthetic antioxidant because it presents a potential free radical scavenging activity similar to those observed for the used reference standard antioxidant products. This potential free radical scavenging ability can be attributed to the active hydrogen donor ability of hydroxyl substitution due probably to the high level of phenolic compounds contained in extracts (Perumal, 2007).

Suggesting R.damascena as a natural source of antioxidants in our study is also supported by pther works from literature (Antoaneta, 2023). These studies demonstrated significant antioxidant

activity. Additionally, methanolic extracts of *O. majorana* have shown a significant potential in mitigating liver damage and oxidative stress in experimental models by enhancing ferric reducing antioxidant power and reducing malondialdehyde (MDA) levels, a marker of lipid peroxidation, thereby supporting its antioxidant efficacy (Antoaneta, 2023).

Both our extract showed a considerable antibacterial activity against the tested bacterial strains. the OM extract demonstrated strong antibacterial activity against both strains, with inhibition zones of 7 mm against *S. Aureus* and 20 mm against *E. Coli*, which was very similar to the values reported by Faozia et al. (2017) et al who indicated zones of 17,5 mm diameter against *E. Coli* and 13,5 mm diameter against *S. Aureus* (Sienkiewicz et al. 2011).

While RD extract exhibited a zone of inhibition measuring 10 mm against *E. Coli* and 8 mm against *S. Aureus*. similar results have been shown in Mounia Chroho et al. (2022) works showing 8 mm against *E. Coli* and 4 mm against *S. Aureus* (Sienkiewicz et al. 2011).

The flavanoid components have a remarkable activity against several Gram-positive bacteria, such as *Staphylococcus aueus* and Gram-negative, such as *Escherchia coli* (Stahl-Biskup et Saez 2002).

Moreover, Some phenolic acids (gallic, caffeic, and ferulic acids) showed anti-bacterial activity against Gram-positive (5 *curvus and L. monocytogenes* and Gram-negative *lacteria E coil* and *Preudomonas aeruginosa*), These phenolic acids exert their antibacterial effects by disrupting cell membranes, generating oxidative stress, and inhibiting key bacterial enzymes. This makes them promising candidates for developing new antibacterial treatments, especially in the face of increasing antibiotic resistance (Sienkiewicz et al. 2011).

Finally, It is important to note that experimental trials (in vitro) have inherent limitations, particularly regarding their applicability to food allergy and microbiota modulation. These limitations include differences in how substances interact within a controlled environment compared to a living organism, making it challenging to fully predict the effects on human health (Algebaly et al. 2021).

O.majorana and *R.damascena* is known for its high antioxidant properties, which help mitigate oxidative stress. The presence of phenolic compounds like rosmarinic acid, flavonoids, and terpenoids contributes to its antioxidant capacity of this plants has been shown to positively influence gut microbiota by promoting the growth of beneficial bacteria and inhibiting pathogenic bacteria. This modulation can improve gut health and overall well-being (Aytaç 2020).

To sum up, both *R.damascena* and *O.mjorana* are plants with significant medicinal potential, traditionally utilized for various therapeutic purposes. *R.damascena* is renowned for its antioxidant, anti-inflammatory, and antidepressant properties, making it valuable in managing stress, inflammation, and oxidative stress-related conditions. Its essential oils and extracts are commonly used in aromatherapy and skincare, further highlighting its versatility.

O.majorana has been traditionally used for its antibacterial, antithrombin, and antihyperglycemic effects. The plant's bioactive compounds, such as carvacrol and thymol, contribute to its ability to inhibit bacterial growth, prevent abnormal blood clotting, and regulate blood sugar levels, thus supporting cardiovascular and metabolic health. However, despite their historical and cultural significance, the bioactivity and phytochemical composition of these plants are not fully understood. This underscores the need for further research, including toxicity assessments of their aqueous and methanolic extracts, to ensure their safety and efficacy. Such studies are crucial, given the limitations of in vitro experimental trials, particularly in predicting real-world effects on food allergies and microbiota modulation.

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Conclusions and perspectives

Plants with anti-oxidant and antibacterial properties are considered as a potential source of new alternative drugs with less side effects and low cost. Our work concerning the impact of the *O. majorana* and *R. damascena* on different *in vitro* activity. OM and RD showed an important phenolic and flavonoid content and displayed a remarkable antioxidant and antibacterial activity *in vitro.*

We hypothesized that this effect may be explained by the rich phytochemical composition of our extracts in terms of polyphenols and flavonoids. the phenolic and flavonoid compounds found in *O. majorana* and *R. damascena* extracts possess significant therapeutic potential for managing food allergies and modulating the gut microbiota. The antioxidant activity is evidenced by the ability to scavenge free radicals and reduce oxidative stress, which is crucial in mitigating inflammatory responses associated with food allergies.

The antibacterial activity of these extracts has shown efficacy against a range of pathogenic bacteria, suggesting a role in maintaining a balanced gut microbiota by inhibiting harmful bacterial growth. These properties may collectively contribute to their beneficial effects, enhancing gut health and alleviating allergic reactions.

Nevertheless, large-scale trials are needed to comprehensively understand the mechanism of action of this extract. This includes *in vitro, in vivo,* and clinical experiments to gain insights into its bioavailability and its effects on different stages of the pathology. Further research should focus on the appropriate application of these plant-derived extracts as alternative treatments for food allergies and gut microbiota. The following techniques can be explored in the future.

- Further clinical studies are needed to confirm the efficacy and safety of *O.majorana* and *R.damascena* in modulating gut microbiota and managing food allergies. Understanding the specific mechanisms by which these plants influence the gut-immune axis will provide deeper insights and pave the way for developing targeted therapies
- Enhancing antioxidant defenses and reducing gut inflammation.
- Modulating gut microbiota composition towards a more balanced state.
- Supporting the integrity of the intestinal barrier, thereby preventing allergen translocation.
- Promoting immune tolerance through the production of beneficial metabolites by gut bacteria.

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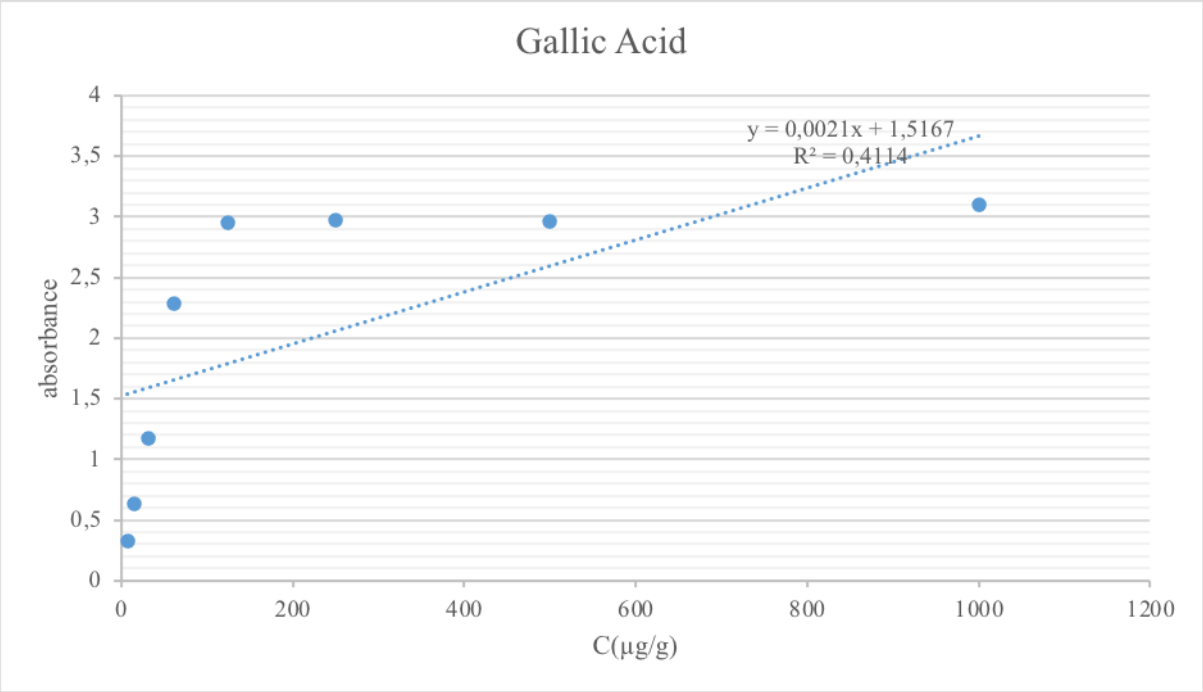
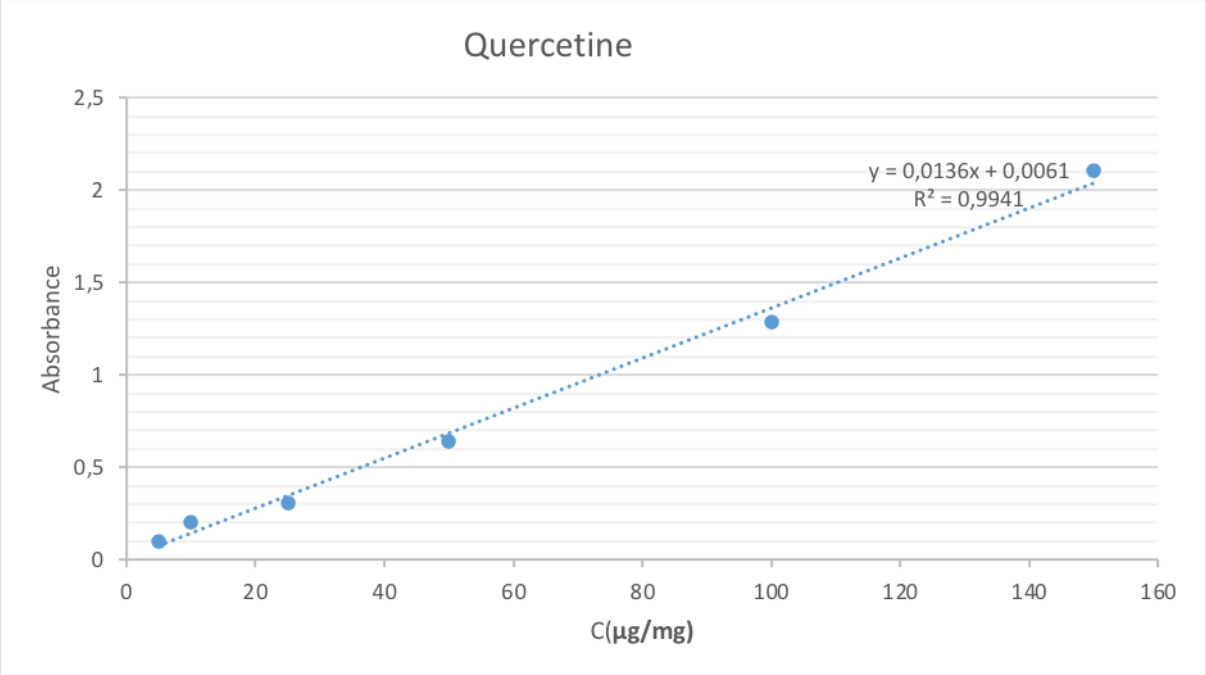
Appendices

Appendices

I. Calibration curve of Gallic acid



II. Calibration curve of Quercetin



Abstracts

Résumé

Dans ce présent travail, *Origanum majorana.L* et *Rosa damascena* sont étudiés pour leur effet potentiel modulateur sur le microbiote et l'état redox lors de la dysbiose médiée par une allergie alimentaire. Les extraits méthanoliques OM et RD ont été obtenus avec un rendement d'extraction de 3,87 % et de 10,21 % respectivement. Les extraits OM et RD étaient les plus riches en terme de teneur en flavonoïdes (respectivement 85,12±11,03 et 93,50±8,97 µg QE/mg). L'évaluation des phénols totaux a révélé que *O.majorana L* et *R. damascena* présentaient une importante teneur phénolique, en particulier OM et RD (660,33±35,37 et 639,80±106,43 µg QE/g, respectivement). Une activité antioxydante *in vitro* significative a été rapportée pour les extraits OM et RD avec des valeurs de IC_{50} de 2,54 ±0,01, 4,57±0,03 respectivement, prouvant une capacité antioxydante pertinente de notre plante *O.majorana L* et *R.damascena*. Nos deux extraits ont montré une activité antibactérienne considérable contre les souches bactériennes testées. L'extrait OM a démontré une forte activité antibactérienne contre les deux souches, avec des zones d'inhibition de 7 mm contre *S. Aureus* et de 20 mm contre *E. Coli*. Tandis que l'extrait de RD a montré une zone d'inhibition de 10 mm contre *E. Coli* et de 8 mm contre *S. Aureus*. Notre étude a démontré que ces extraits de plantes possèdent de fortes activités antioxydants et antibactériennes. L'activité antioxydant, attestée par leur capacité à neutraliser les radicaux libres et à réduire le stress oxydatif, est essentielle pour atténuer les réponses inflammatoires associées aux allergies alimentaires. Par ailleurs, l'activité antibactérienne de ces extraits a montré une efficacité contre une gamme de bactéries pathogènes, suggérant un rôle dans le maintien d'un microbiote intestinal équilibré en inhibant la croissance des bactéries nocives. Ces propriétés contribuent collectivement à leurs effets bénéfiques, améliorant la santé intestinale et atténuant les réactions allergiques. Nous suggérons *O.majorana* L. et *R.damascena* L. comme source puissante d'agents antioxydants. D'autres essais sont requis afin de mieux élucider leur effet modulateur du système immunitaire lors d'une allergie alimentaire lié à un dysbiose intestinal.

Mots clés *: O.majorana* ; *R .damascena* ; l'allergie alimentaire ; microbiote, ployphénol ; stress oxydatif ; dysbiose

ملخص

في هذا العمل قمنا بتقييم كل من *Origanum majorana L.* و*R. damascena* من اجل دراسة تأثيرها المحتمل في تعديل الميكروبيوتا وحالة الأكسدة والاختزال أثناء الاختلال الجرثومي الناجم عن الحساسية الغذائية. تم الحصول على مستخلصات الميثانول من OM و RD بعائد استخلاص بنسبة 3.87٪ و 10.21٪ على التوالي. كانت مستخلصات OM و RDهي الأغنى من من حيث محتوى الفلافونويد(11,03±85,12 8.97 ± 93.50 ميكروجرام من / QEمجم ، على التوالي) كشفت تقييمات الفينولات الكلية أن *O. majorana* و *R. damascena* تحتويان على محتوى فينولي كبير، خاصة OM و (35.37±660.33 و106.43 **±**639.80 ميكروجرام **QE /** جرام على التوالي **.** قمنا بتحديد النشاط المضاد للأكسدة مخبريا OMو RD بقيم IC_{50} تبلغ 0.01**±**2.54 و 0.03**±**4.57 على التوالي**.** أظهرت كلا المستخلصات نشاطًا مضادًا للبكتيريا ملحوظًا ضد السلالات البكتيرية المختبرة. أظهر مستخلص OM نشاطًا مضادًا قويًا للبكتيريا ضد كلا السلالتين، مع مناطق تثبيط تبلغ 7 مم ضد *S. aureus* و 20 مم ضد*E. coli* ، بينما أظهر مستخلص RD مناطق تثبيط تبلغ 10 مم ضد *E. coli* و 8 مم ضد *S. aureus* أظهرت دراستنا أن هذه المستخلصات النباتية تمتلك نشاطات مضادة للأكسدة ومضادة للبكتيريا قوية. إن النشاط المضاد للأكسدة، الذي يتجلى في قدرتها على تحييد الجذور الحرة وتقليل الإجهاد التأكسدي، ضروري لتخفيف الاستجابات الالتهابية المرتبطة بالحساسية الغذائية. علاوة على ذلك، أظهر النشاط المضاد للبكتيريا لهذه المستخلصات فعالية ضد مجموعة من البكتيريا المسببة للأمراض، مما يشير إلى دور في الحفاظ على ميكروبيوتا الأمعاء المتوازنة عن طريق تثبيط نمو البكتيريا الضارة. تسهم هذه الخصائص مجتمعة في فوائدها، مما يحسن صحة الأمعاء ويخفف من ردود الفعل التحسسية. إذن نقترح *O.majorana L* و *R.damascena* كمصدر فعال لعوامل مضادات هناك حاجة إلى مزيد من التجارب لتوضيح تأثيرهما المعدل لجهاز المناعة أثناء حساسية غذائية مرتبطة بتوازن البكتيريا في الأمعاء.

الكلمات المفتاحية: *R.damascena* ; *O.majorana L* **;** مرض الحساسية الغداية ; الميكروبيوتا ; البوليفينول ; الإجهاد التأكسدي ; الامعاء**.**

Academic year: 2023/2024	**Presented by: Ms. Bezziche Mélissa Rania**

The modulatory effect of *Origanum majorana (Lamiaceae)* and *Rosa damascena* (Rosaceae) on microbiota and redox-state during dysbiosis-mediated food allergy: bibliographic and experimental study.

Thesis submitted for the obtention of Master degree in Biochemistry

Herein, *Origanum majorana L.* and *Rosa damascene* are investigated for their potential modulatory effect on microbiota and redox state during dysbiosis mediated food allergy. Extraction was carried out using the ultrasonification method. The tested plants were assessed for their phenolic compositio, antioxidant activity *in vitro,* and antibacterial activity. The antioxidant activity was evaluated using the DPPH method, which clearly demonstrated the biological potential of these plants and could justify their use in traditional medicine. Antibacterial activity was assessed using the well diffusion method. Methanolic extracts of OM and RD were obtained with extraction yields of 3.87% and 10.21%, respectively. Evaluation of total phenols revealed that both *O.majorana* and *R.damascena* demonstrated an important phenolic content OM and RD showing the highest amounts (660.33±35.37 and 639.80±106.43 µg QE/g, respectively). OM and RD, while being the richest in terms of phenols, also had the highest flavonoid content, measuring 85.12±11.03 and 93.50±8.97 µg QE/mg, respectively. a significant *in vitro* antioxidant activity was reported for OM and RD extracts, with IC_{50} values of 2.54±0.01 and 4.57±0.03, respectively, demonstrating the relevant antioxidant capacity of *O.majorana* and *R. damascena.* Both our extract showed a considerable antibacterial activity against the tested bacterial strains. the OM extract demonstrated a strong antibacterial activity against both strains, with inhibition zones of 7 mm against *S. Aureus* and 20 mm against *E. Coli*, While RD extract exhibited a zone of inhibition measuring 10 mm against *E. Coli* and 8 mm against *S. Aureus*. Our study has demonstrated that these plant extracts possess strong antioxidant and antibacterial activities. The antioxidant activity, evidenced by their ability to scavenge free radicals and reduce oxidative stress, is essential for mitigating inflammatory responses associated with food allergies. Furthermore, the antibacterial activity of these extracts has shown efficacy against a range of pathogenic bacteria, suggesting a role in maintaining a balanced gut microbiota by inhibiting harmful bacterial growth. These properties collectively contribute to their beneficial effects, improving health and alleviating allergic reactions. We suggest *O.majorana* and *R.damascena* as potent source of antioxidant agents. Nevertheless, further assays are needed to better elucidate their actions on the immune system and food allergies mediated by dysbiosis.

Mots-clefs *:* *O.majorana L; R .damascena*; food allergies; gut microbiota; polyphenols; dysbiosis; oxidative stress

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