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In vivo study of the anti-inflammatory effect of natural extracts from *Cynara scolymus* (Artichoke) bracts and *Citrus sinensis* (Sweet orange) peels by-products

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Summary

In light of the 190 million tons of fruit and vegetable by-products generated annually worldwide, and the need for sustainable waste valorization strategies, this study investigates the anti-inflammatory potential of two such by-products: artichoke bracts (*Cynara scolymus*) and sweet orange peel (*Citrus sinensis*). A hydro-alcoholic extraction was performed for artichoke bracts and an aqueous extraction for orange peels. These plants are rich in polyphenols and flavonoids known for their biological activities. The anti-inflammatory effect of their extracts was evaluated *in vivo* using male *Wistar albino* rats through three well known of acute inflammation models: xylene-induced ear edema, (1%) λ-carrageenan-induced paw edema, and peritonitis. In the xylene-induced ear edema model, the orally administered artichoke bracts extract (ABE) and orange peel extract (OPE) at a similar dose of (500mg/kg) significantly ($P<0.05$) exhibited strong inhibition rates of 71.8% and 70.2% respectively, while the standard reference aspirin administrated at a dose of (400mg/kg) showed 64.6%. In the paw edema test, they showed up to 82% and 67% inhibition, respectively, with clear reduction in swelling. In the peritonitis model, both extracts significantly inhibited neutrophil migration reaching 87.2% (ABE) and 86.6% (OPE), similar to aspirin (85.8%). These findings suggest that both extracts have potent anti-inflammatory properties and could serve as effective, natural alternatives to conventional drugs like aspirin.

Keywords: by-product, **v**alorization, polyphenols, anti-inflammatory, inflammation, orange peels, Artichoke bracts.

Resumé

À la lumière des 190 millions de tonnes de sous-produits de fruits et légumes générés chaque année dans le monde , et face au besoin croissant de stratégies durables de valorisation des déchets, cette étude vise à évaluer le potentiel anti-inflammatoire de deux de ces sous-produits : les bractées d'artichaut (*Cynara scolymus*) et la peau d'orange douce (*Citrus sinensis*). Une extraction hydro-alcoolique a été réalisée pour l'artichaut, et une extraction aqueuse pour Lorange. Ces derniers sont riches en polyphénols et flavonoïdes, connus pour leurs activités biologiques. L'effet anti-inflammatoire de leurs extraits a été évalué *in vivo* chez des rats mâles de l'espèce *Wistar albinos*, à travers trois modèles classiques d'inflammation aiguë : l'œdème de l'oreille induit par le xylène, l'œdème de la patte et la péritonite induite par le (1%) λ-carraghénane. Dans le modèle d'œdème de l'oreille, les extraits de bractées d'artichaut (ABE) et de la peau d'orange (OPE) administrés par voie orale a une dose de (500mg/kg) significativement ($P<0.05$) ont montré des taux d'inhibition élevés, atteignant respectivement 71,8 % et 70,2 %, tandis que la substance de référence l'aspirine administrée à une dose de (400mg/kg) a montré un pourcentage d'inhibition de 64,6 %. Dans le modèle d'œdème de la patte, les taux d'inhibition ont atteint jusqu'à 82 % pour ABE et 67 % pour OPE, avec une réduction claire et significative de l'enflure. Concernant la péritonite, les deux extraits ont significativement inhibé la migration des neutrophiles, atteignant 87,2 % pour ABE et 86,6 % pour OPE, des résultats comparables à ceux de l'aspirine (85,8 %). Ces résultats suggèrent que les extraits des deux sous-produits naturels possèdent des propriétés anti-inflammatoires puissantes, et pourraient constituer des alternatives naturelles et efficaces aux médicaments conventionnels comme l'aspirine.

Mots-clés: Sous-produit, valorisation, polyphénols, inflammation, anti-inflammatoire, bractées d'artichaut, peau d'orange.

ملخص

في ضوء الكمية الكبيرة الناتجة عن مخلفات الفواكه والخضروات والتي تقدّر بحوالي 190 مليون طن سنويًا على مستوى العالم، وفقًا لمنظمة الأغذية والزراعة، والحاجة المتزايدة لإيجاد طرق مستدامة للاستفادة من هذه النفايات، تهدف هذه الدراسة إلى تقييم التأثير المضاد للالتهاب لاثنين من هذه المخلفات: قنابات الخرشوف الارضي (*Cynara scolymus*) وقشور البرتقال الحلو.(*Citrus sinensis*) تم إجراء عملية استخلاص باستخدام الكحول للخرشوف، في حين تم استخدام الماء لاستخلاص البرتقال. تُعدّ هذه النباتات غنية بالبوليفينولات و الفلافونويدات المعروفة بنشاطها البيولوجي. وقد تم تقييم التأثير المضاد للالتهاب لمستخلصاتهما باستخدام ذكور فئران التجارب من فصيلة ألبينو ويستار، عبر ثلاث نماذج معروفة للالتهاب الحاد؛ وذمة الأذن الناتجة عن مادة الزيلين، وذمة القدم والتهاب الصفاق الناتجان عن الكاريجنان . حيث في نموذج وذمة الأذن، أظهر مستخلص أوراق الخرشوف (ABE) ومُستخلص قشور البرتقال (OPE)المُعطاة عن طريق الفم بجرع(500ملغ/كغ) معدلات تثبيط قوية بلغت 71.8% و70.2% بالترتيب، في حين أن المرجع، الأسبرين المُعطى بجرع(400 ملغ/كغ) ، أظهر معدل 64.6%. أما في اختبار وذمة القدم، فقد وصلت نسبة التثبيط إلى 82% لـ ABE و67% لـOPE ، مع انخفاض واضح في الانتفاخ. وفي نموذج التهاب الصفاق، سجل كلا المستخلصين تثبيطًا كبيرًا لهجرة العدلات بنسبة 87.2% لـ ABE و86.6% لـOPE ، وهي نتائج قريبة جدًا من فعالية الأسبرين التي تقدر بنسبة 85%. تشير هذه النتائج إلى أن كلا المستخلصين يتمتعان بخصائص قوية مضادة للالتهاب، وقد يشكلان بدائل طبيعية وفعّالة للأدوية التقليدية مثل الأسبرين.

الكلمات المفتاحية: ناتج ثانوي، تثمين، بوليفينولات، التهاب، مضاد التهاب، قنابات الخرشوف الارضي الشوكي، قشور البرتقال.


بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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- **ABE:** Artichoke Bracts Extract.
- **ABTS:** 2, 2'-azino –bis (3-ethylbenzothiazoline-6-sulfonic acid).
- **CCL:** Chemokine (C-C motif) Ligands.
- **CXCL:** Chemokine (CXC motif) Ligands.
- **COX:** Cyclooxygenase.
- **DAMPs:** Damage – Associated Molecular Patterns.
- **DPPH:** 2, 2-diphenyl -1-picrylhydrazyl.
- **FRAP:** Ferric Reducing Antioxidant Power.
- **GM-CSF:** Granulocyte-Marcophage Colony-Stmulating Factor.
- **HMG –CoA :** 3-Hydroxy-3-Methylglutaryl-Coenzyme A.
- **IBD:** Inflammatory Bowel Disease.
- **i NOS:** inducible Nitric Oxide Synthase.
- **IFN-γ:** Interferon – Gamma.
- **MBP:** Mannose-Binding Protein.
- **MAPK:** Mitogen – Activated Protein Kinase.
- **NETs:** Neutrophil Extracellular Traps.
- **NF-κB:** Nuclear Factor Kappa - light-chain enhancer of activated B cells.
- **OPE:** Orange Peel Extract.
- **PAF:** Platelet -Activating Factor.
- **PAMPs:** Pathogen- Associated Molecular Patterns.
- **PMFs**: Polymethoxylated Flavones.
- **ROS:** Reactive Oxygen Species.
- **SLPI :** Secretory Leukocyte Protease Inhibitor.
- **STLs:** Sesquiterpene Lactones.
- **TGF-ß:** Transforming Growth Factor -Beta.
- **TNFα:** Tumor Necrosis Factor Alpha.

General Introduction

Introduction

According to the Food and Agriculture Organization statistics **(FAO, 2022)**, industrial food processing and daily human consumption of fruits and vegetables generate considerable amounts of by-products approximately 190,000 Kt annually, including leaves, bracts, seeds, peels, and spines. Improper disposal of these wastes poses significant environmental challenges, such as water and soil pollution **(Diluca et al., 2020; Colombo et al., 2024)**. Furthermore, conventional waste management techniques like incineration release harmful pollutants, while landfilling contributes to greenhouse gas emissions, including methane and carbon dioxide, thereby increasing environmental and health risks **(Nirmal et al., 2023)**. Traditional uses of these by-products, such as animal feed or biomass production, are insufficient to limit their accumulation. Therefore, valorizing fruit and vegetable wastes into high-value secondary products offers promising solutions to environmental, economic, and food security issues **(Usman et al., 2025)**. Among the valuable compounds found in these by-products are polyphenols, flavonoids, and vitamins, which have demonstrated diverse biological activities **(Joshi et al., 2020; Hano & Tungmunnithum., 2020)**.

One critical health concern where such natural compounds show potential is inflammation **(Sun et al., 2024)**. It is a vital physiological process that protects the organism from pathogens and repairs tissue damage. However, excessive or uncontrolled inflammation can damage host tissues and lead to severe chronic disorders and organ failure **(Riaz & Sohn, 2023)**. While current anti-inflammatory drugs target specific steps in the inflammatory pathway, their use is often limited by side effects such as metabolic syndromes, stomach irritation, depression, indigestion, and menstrual irregularities **(Calhelha et al., 2023)**.

Given these challenges, recent pharmaceutical research has increasingly focused on natural anti-inflammatory agents derived from plants, foods, and spices, aiming to develop safer and more effective therapies with minimal side effects **(Leri et al., 2020)**.

Building on the potential of fruit and vegetable by-products as rich sources of bioactive compounds, this study focuses on evaluating the *in vivo* anti-inflammatory effects of extracts derived from sweet orange peels and artichoke bracts. These by-products, often discarded as waste, contain valuable polyphenols, flavonoids, and vitamins that may offer safer and multi-targeted alternatives to synthetic anti-inflammatory drugs. The objective of our study is to investigate the efficacy of these extracts in modulating acute inflammation in animal models, thereby contributing to sustainable waste valorization and the development of natural therapeutic agents for chronic inflammatory disorders.

Literature review

Chapter 01: The inflammatory response

I Inflammatory response

Inflammation is a fundamental physiological process and one of the sophisticated innate immunity responses. It is highly regulated against injury and infection and it preserves tissue homeostasis under harmful conditions. Historically, the earliest evidence of inflammation has been identified in dinosaur bones. It has affected humans since their early existence, as seen in the bones of early ancestors and *Homo sapiens* **(Cavaillion, 2021).** Etymologically, the word "Inflammation" is derived from the Latin "*Inflammare*" which means: to ignite, to set alight or burn. The comparison to fire is illustrative, as the purpose of inflammation from a teleological perspective, is to initiate a defensive response against various invaders and to naturally attenuate after threats clearance **(Oronsky et al,. 2022).**

1.1 Signs of inflammation

Around 2000 years ago, Aulus Cornelius Celsus a Roman encyclopedist was one of the firsts to define the four cardinal signs of inflammation which are still relevant in modern medicine: redness (*rubor*), swelling (*tumor*), heat (*calor*), pain (*dolor*) **(Stone, 2017)**.

- Redness and heat: result from increased blood flow to the affected area.
- Swelling: caused by fluid accumulation.
- Pain: results from the release of substances that stimulate nerve endings. **(Hannoodee & Nasruddin, 2024).**
- In the 19th century, Rudolf Virchow added the loss of function (*functio laesa*) **(Liu et al., 2017).**

1.2 The inflammatory response components

The inflammatory process is composed of four main elements that work together to initiate and regulate the body's defense mechanism. They include:

1.2.1 Inflammatory inducers

The inflammation inducers are factors that activate the inflammatory pathway classified into:

- **Physical:** Burn, frostbite, physical injury, trauma, lionizing radiation, irritants, and large foreign bodies (silica, asbestos) **(Hannoodee & Nasruddin, 2024)**.
- **Chemical:** glucose **(Tchivhase et al., 2024)**, fatty acids, toxins, alcohol, chemical irritants (including fluoride, nickel and other trace elements).
- **Biological:** signals from damaged cells **(Chen et al., 2017).**

- **Psychological:** stress which can significantly increase inflammatory activity even without physical injury for example social stressors; they are powerful activators of systemic inflammation. Some studies have confirmed that negative social interactions are linked to increased levels of inflammatory markers **(Slavich & Irwin, 2014)**.
- **Infectious factors:** Bacteria, viruses and other microorganisms.
- **No infectious factors:** Allergens **(Hannoodee & Nasruddin, 2024).**

1.2.2 Inflammatory sensors

Inflammatory sensors play a pivotal role in detecting microbial invasion and tissue injury. They serve as the primary mediators of immune surveillance and are expressed on phagocytes, dendritic cells, and various other cell types, including lymphocytes, epithelial and endothelial cells. These receptors termed "Pattern-recognition receptors (PRRs)" for their ability to identify conserved molecular patterns in pathogens, known as (PAMPs), and endogenous signals released from damaged cells, termed (DAMPs) **(Abbas & Litchtman, 2011)**. Upon activation, PRRs trigger signaling events such as the MAPK and NF-κB pathways, which result in the production of pro-inflammatory cytokines and chemokines **(Zivkovic et al., 2021)**. They are located in different cellular compartments (**Figure 1**), and based on their structure, ligand specificity, and activated signaling pathways PRRs are classified into families: RIG-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and Toll-like receptors (TLRs) **(Gosh, 2017)**.

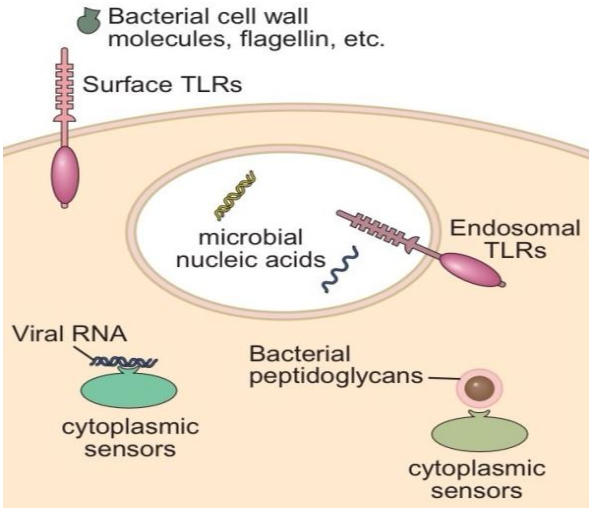

Figure 1 TLRs distribution
(Abbas & Litchtman, 2011)

1.2.3 The inflammatory cells

There are a collection of inflammatory cells represented in (**Table 1**). While some play key immunological functions others contribute to this process as an accessory and complementary elements **(Wang et al., 2020)**.

Table 1: Cells involved in the inflammatory process

cells	Basic role in inflammation	References
Macrophages	Macrophages are plastic immune cells that can switch their phenotypes and function in response to environmental signals: - **M1 (Pro-inflammatory) macrophages** initiate and sustain inflammation, secrete pro-inflammatory cytokines, activate endothelial cells and enhance recruitment of other immune cells to the site of inflammation. - **M2 (pro-resolving) macrophages** serve help to resolve inflammation by releasing anti-inflammatory mediators, phagocytosis of apoptotic cells, collagen deposition and tissue repair.	**(viola et al., 2019)**
Neutrophils	Neutrophils are the first leucocytes to arrive at sites of infection, initiating inflammation. They combat pathogens through phagocytosis, release of reactive oxygen species, granular enzymes, and formation of neutrophil extracellular traps (NETs). Neutrophils also produce cytokines that regulate inflammation and coordinate recruitment and activation of other immune cells like macrophages and monocytes**.**	**(Rosales., 2018)**
Eosinophils	Eosinophils have multiple functions they contribute to protective immune response, tissue repair and regeneration. Eosinophils also have a role in pathological inflammation. Their recruitment is driven by mediators (IL-5, Il-4, IL-13 and eotaxin), once activated eosinophils release toxic granules and pro-inflammatory mediators.	**(Lombardi et al., 2022)**
Mast cells	Mast cells are one of the key inflammatory cells that drive inflammation by releasing mediators like vasoactive substances, cytokines and proteases.	**(Theoharides et al.,2012)**
Platelets	Platelets play a crucial role in inflammation by acting beyond their traditional function in clotting. They accumulate at sites of tissue damage and adhere to white blood cells, releasing cytokines and chemokines that attract neutrophils, monocytes, and lymphocytes to the inflammation site.	**(Sonmez, O, & Sonmez, M., 2017).**

Fibroblasts	They are principle cells in wound healing and fibrosis. In response to signals fibroblasts activate immune cells including leukocytes and promote their recruitment by releasing cytokines, and chemokines.	**(Buechler, & Turley.,2018) (Gauthier et al.,2023)**
Endothelial cells	In their resting state they preserve vascular homeostasis by controlling blood flow and maintaining anti-inflammatory surface. Once they are activated, endothelial cells regulate leukocyte adhesion and migration by expressing adhesion molecules (E-selectin, ICAM, VKAM) which facilitate leukocyte extravasation.	**(Neubauer, & Zieger., 2022)**
lymphocytes	Inflammation signals increase lymphocyte recruitment to lymph nodes, triggering adaptive immune responses. Activated T lymphocytes release cytokines to enhance immune activity, while B lymphocytes produce antibodies that neutralize pathogens and help in their clearance.	**(Moro-García.,2018)**

1.2.4 The inflammatory mediators

Inflammatory mediators are a group of molecules and signal proteins classified in the **(Table 2)** that regulate the initiation and resolution of inflammation. Their coordinated interactions are essential for producing an effective and balanced inflammatory response **(King, 2007).**

Table 2: Classification of inflammatory mediators (King, 2007; Abdulkhaleq et al., 2018).

Class	**Molecule**	**Function**	**Origin**
Cytokines	Proinflammatory : (IL)-1β, IL-8, IL-6, IL-12, IL-17, TNF-α and IFNγ.	- Enhance vascular permeability - Boost the outcome of other inflammatory mediators - Increase the blood flow and plasma exudation resulting in edema	Monocytes Fibroblasts Endothelial cells Epithelial cells Dendritic cells Th cells
	Anti-inflammatory : IL-4, l'IL-10, and the TGFβ	- Decreasing the pro-inflammatory cytokines secretion - Attenuating the signaling pathways of certain inflammatory sensors (IL-10 has been shown to	

		inhibit 10 to 15% of genes activated through TLR signaling) **(Medzhitov, 2010).** - Resolution and tissue repair.	
Acute-phase Proteins	Complement component C3 (C3a and C3b)	- Chemotactic for (neutrophils) - Increasing the vascular permeability. - Activation of histamine release **(Krishnappa ,2016).**	Hepatocytes
	Mannose-binding protein (MBP)	- Facilitates pathogen recognition and opsonization.	
	C-reactive protein (CRP)	- Boosting the opsonization. - Activating the complement system. - Stimulating cytokines production. - Inhibiting chemotaxis.	
	Serum amyloid A	- Chemiotaxis. - Anti-inflammatory activity.	
	Fibrinogen	- Tissue repair. - Fibrinopeptides generation and clotting.	
	Haptoglobin	- Bacteriostatic (limiting the metabolism of bacteria by decreasing iron levels).	
vasoactive amines and peptides	Histamine	- Enhance PG synthesis and induce localized pain. - Promote vasodilation and vascular permeability.	Basophils
	Serotonin		platelets Basophils
	Bradykinin		Plasma Kinin–Kallikrein system

Free radicles	NO (nitric oxide)	- Enhances the antimicrobial activity of macrophages **(viola et al., 2019).** - Regulation of inflammatory cytokines production **(Kobayashi,2010).**	Mast cells, Macrophages and Endothelial cells
Lipid-derived mediator	PAF	- Platelets aggregation. - Induces leukocytes recruitment **(Singh et al.,2013).**	Platelets, Macrophages, Eosinophils, Basophils, and Endothelial cells
	Thromboxanes	- Activation, aggregation and degranulation of platelets. - Increases vascular permeability. - Increasing the expression of leucocyte adhesion molecules) **(Mendoza et al.,2021**).	Platelets and Endothelial cells
	Prostaglandins (PGE2;PGb)	- Stimulate the release of Pro-inflammatory cytokines (IL-8) and induce anti-inflammatory cytokines secretion IL-10) **(Sheppe &Edelmann,. 2021).** - Increase vascular permeability, vasodilatation **(Krishnappa, 2016)** - Activate platelet aggregation **(Diallo, 2019).**	Macrophages, monocytes, neutrophils and mast cells.
	Leukotrienes	- Activate immune cells recruitment. And increase their phagocytosis and microbicidal activity. - Modulates the production of chemokines and cytokines **(Mendoza et al.,2021).**	Rhogocytes, Necrophages, lymphocytes, Neutrophils and Eosinophils

1.3 Types of inflammation

Inflammation can exhibit either therapeutic or pathological characteristics (**Figure 2**), depending on various factors. It can be protective, termed acute inflammation, which play a crucial role in preventing tissue injury and fighting infection. In contrast, under uncontrolled or excessive conditions, inflammation can be destructive, hindering the normal homeostatic process and becomes pathological leading to the progression of various diseases. This type is termed chronic inflammation **(Arulselvan et al., 2016)**.

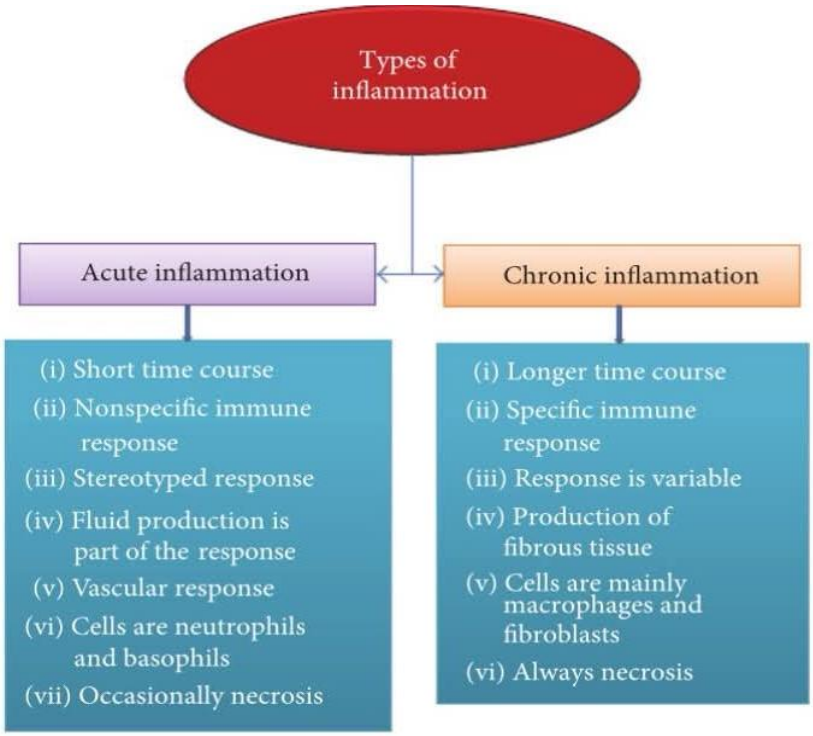

Figure 2: Classification of inflammation **(Arulselvan et al., 2016)**

1.3.1 Acute inflammation

Acute inflammation is a rapid and short-term response that occurs within minutes and can last for hours following tissue damaged caused by trauma, toxins or infection **(Abd Hafid & Iran, 2019)**. The stages of acute inflammation are:

a) **Vascular phase:** This phase is characterized by vascular dilation, hyperemia, exudation and pain. The release of small signaling molecules such as anaphylatoxins at the site of infection **(Abdul Hafid & Iran, 2019)**, which activate the secretion of mediators that produce endothelial contraction (histamine, serotonin, bradykinin and PAF). And altering vascular endothelial permeability (histamine, NO, and prostaglandins). That leads to plasma proteins influx such as antibodies and other components, and allows the recruitment of leucocytes to the site of infection **(Krishnappa, 2016)**.

b) Cellular phase: Neutrophils are the first cell type recruited to the site of infection by a process called diapedesis (**Figure 3**). This process is strongly regulated by adhesion molecules (selectins, integrins and cadherins) present on the cell surface of both; the leukocytes and the endothelium cells **(Su et al., 2020)**. Once at the site of infection, neutrophils act to eliminate pathogens through effective mechanisms including phagocytosis, ROS production, degranulation and NETs release. Malfunction or hyperactivity of neutrophils during the acute inflammation process may cause acute inflammatory diseases such as stroke **(Xing et al., 2012)** and sepsis **(Nedeva, 2021)**.

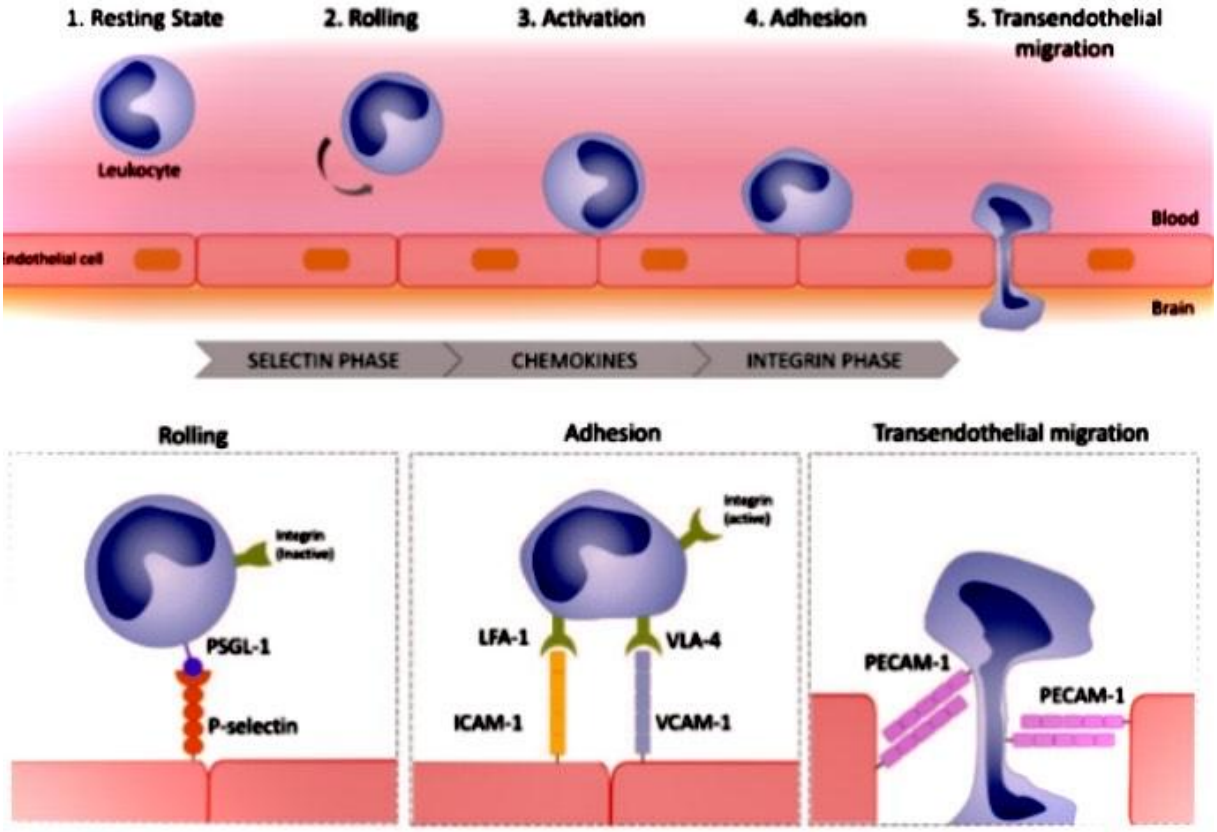

Figure 3: schematic representation of the multistep diapedesis process **(Gauberti et al., 2018)**

c) Resolution phase: It plays a crucial role in terminating the inflammation (**Schett & Neurath,2018)**. It is self-limited and highly regulated process. This phase is characterized by the entrance of mononuclear cells, monocytes and macrophages to the site of inflammation in order to clear the cellular debris and apoptotic neutrophils by the phenomena of phagocytosis (**Freire & Van Dyke., 2013)**. In addition to the precedence of anti-inflammatory and pro-resolving factors over pro-inflammatory mediators **(Perucci et al., 2017)**. This imbalance is regulated by inhibiting the pro-inflammatory signaling pathways, boosting anti-inflammatory cytokines secretion and activating

regulatory cells **(Calder et al, 2013)**. The failure of resolution process can lead to prolonged tissue damage and the development of chronic inflammatory diseases.

1.3.2 Chronic inflammation

Chronic inflammation is a prolonged immune response that persists for days to weeks. It often develops during the transition from acute inflammation to tissue repair and is characterized by a predominantly mononuclear inflammatory cell infiltrate, with few neutrophils **(King, 2007)**. Disruption of balance between pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNFα) and anti-inflammatory cytokines (IL10, IL-4, IL-13 and TGFβ) is also observed. Chronic inflammation arises from the persistent presence of specific inciting substances and agents including infections and continuous chemical exposures such as silicates and grass awns that continuously stimulate the immune system **(Ackermann, 2017)**. When this inflammatory response becomes sustained and unregulated, it can lead to progressive tissue damage. Over time, such unresolved inflammation contributes significantly to the development and advancement of various chronic diseases. The key factors implicated in the progression of chronic inflammatory conditions are represented in the following (**Table 3**).

Table 3: factors contributing to the development of chronic inflammation and diseases

Factor	Description	Diseases	References
Smoking	Stimulate the production of pro-inflammatory cytokines and reduce the level of anti-inflammatory cytokines.	– Cancer – Chronic lung disorders – Vascular diseases	**(Chavada et al., 2024)**
Obesity	Activation of pro inflammatory cells and cytokines release.	– Atherosclerosis – Type 2 diabetes	**(Savulescu-Fiedler et al., 2024)**
Aging	Induction of oxidative damage, free radicals accumulation, mitochondrial dysfunction, Pro-inflammatory lymphokines up-regulation and immune cells depletion.	– Sarcopenia – Parkinson's disease – Atherosclerosis – Type 2 diabetes	**(Ventura et al.,2017)**
Diet	Deficiency in some nutriments (thiamine), poor nutrition, unhealthy aliments (refined sugar, saturated fats and trans-fats) increase the risk of chronicity of inflammation	– Cardiovascular diseases – Type 2 diabetes – Inflammatory bowel disease (IBD) – Rheumatoid arthritis	**(Wagenaar and al.,2021)**

1.4 Treatment of inflammation

Inflammation can be effectively treated and managed using pharmaceutical drugs (NSAIDs SAIDs):

1.4.1 Non-steroidal anti-Inflammatory drugs

NSAIDs are a class of chemical compounds primarily employed as first-line therapeutics for the management of inflammation, pain, and fever. Their principle mechanism of action involves the blocking of prostaglandins synthesis (**Figure 4**) by inhibiting the activity of PG biosynthetic enzymes (COX-1 and COX-2).

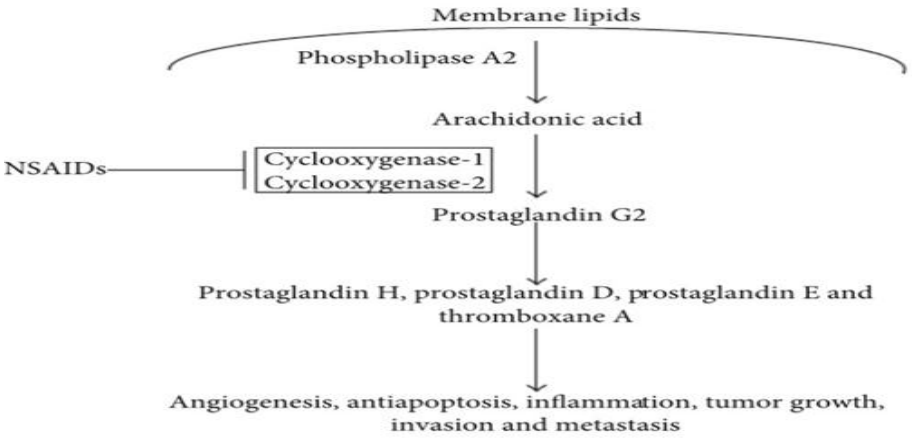

Figure 4: schematic of the mechanism action of NSAIDs **(Ishiguro, & Kawahara.,2014)**

While COX-2 expression is inflammatory cytokines and GFs dependent COX-1 is ubiquitous and expressed in most tissues playing important roles maintaining hemostasis and blood clotting. NSAIDs are classified into two subclasses (**Table 4**) COX-2 non-selective NSAIDs which inhibit both COX-1 and COX-2, and COX-2 selective NSAIDs which inhibit COX-2 only **(Ishiguro, & Kawahara., 2014)**.

Table 4: classification of NSAIDs and their COX-1/COX-2 selectivity (Kuritzky & Samraj., 2012; Park & Bavry., 2014, Mügge et al., 2015)

Chemical class	COX-1 selective NSAIDs	COX-2 selective NSAIDs
Salicylate	Aspirin	-
Acetic acid derivatives	Indomethacin	Diclofenac
Propionic acid derivatives	Ibuprofen	-
Anthranilic acid (Fenamate)	-	Mefenamic acid
Enolic acids (Oxicams)	Meloxicam	Piroxicam
Sulfonanilide	-	Nimesulide

Diarylheterocycles (Coxibs)	-	Celecoxib Parecoxib Valdecoxib

1.4.2 Glucocorticoids (SINDs)

Glucocorticoids are steroid hormones which play several roles in different physiological processes including inflammation. The naturally short biological half-life of endogenous corticosteroids has driven the development of synthetic glucocorticoid analogs such as prednisone, prednisolone, methylprednisolone, triamcinolone, dexamethasone, and betamethasone **(Yang & Yu., 2021)**. These cortisone derivatives are designed to prolong their pharmacokinetic and enhance their efficacy. Their role as anti-inflammatory drugs is manifested through their molecular action in the nucleus represented in (**Figure 5**) by switching on genes encoding anti-inflammatory cytokines, MAPK phosphatase-1 which inhibits MAPK cascade and SLPI which is responsible of serine proteases inhibition such as neutrophil elastase, and the protection from tissue damage **(Barnes.,2011)**. In the other hand Glucocorticoids switch off genes encoding pro-inflammatory cytokines including IL-6, IL-2, IL-3, IL-4, IL-5, TNFα, GM-CSF, CCL1, CCL5, CCL11, and CXCL8 **(Yang & Yu ., 2021)**

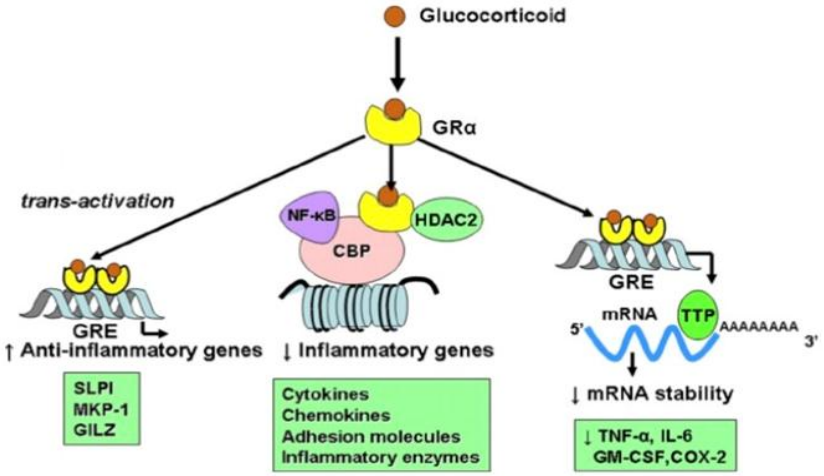

Figure 5: the anti-inflammatory effect of glucocorticoids **(Barnes., 2011)**

Literature review

Chapter 02: selected plant species

II Globe artichoke (*Cynara cardunculus var. scolymus L.*)

Cynara cardunculus var. scolymus L. known as Artichoke is an ancient plant acknowledged as food for its nutritional benefits, and as a medicine for its therapeutic properties which may be due to the variety of its phytochemical composition. Artichoke and its products have garnered increasing attention in scientific research due to their potential biological activities, as demonstrated in various *in vivo* and *in vitro* studies **(Olas, 2024)**.

2.1 Geographical distribution

Cynara scolymus, the globe artichoke, is mainly grown in the Mediterranean region, the Canary Islands, Egypt, and parts of Asia and South America **(Olas, 2024)**. The artichoke's origin and spread are often linked to the Arabs, who, during their dominance of the southern Mediterranean in the Middle Ages, played a significant role in introducing and diffusing this crop **(Sonnante et al,. 2007)**. Artichoke is currently cultivated and produced in many countries around the world as reported by the FAO, the global production of artichoke in 2022 was (1,584,513 tons), with Italy (376,280 tons), Egypt (315,408 tons), Spain (214,560 tones), Algeria (120,932 tones), and Peru (99,518 tones) which represent the world's leading producers **(stea et al.,2023)**.

2.2 Morphological description

The artichoke is a tall plant reaching up to 150-180 cm, with large, light green leaves that are lance-shaped, growing between 50 and 200 cm long. Each plant typically yields one large primary flower head (the choke) which contains the edible part (receptacle) at the apex of the stem (**Figure 6**) along with 4 to 20 smaller secondary and tertiary buds on the branches **(Feiden et al., 2023)**.

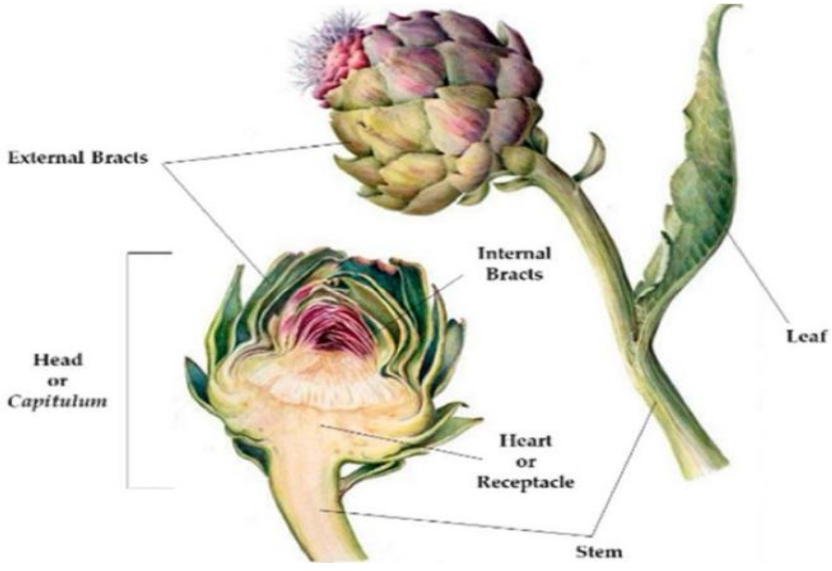

Figure 6: The artichoke's morphology **(Ayuso et al., 2024)**

2.3 Botanical classification of *C. scolymus*

The artichoke, *Cynara cardunculus var. scolymus L.* is a perennial thistle in the *Asteraceae* family, which includes over 2,000 species **(Porro, 2024; Olas, 2024)**. The botanical classification of the plant is represented in the following **(Table 5)**.

Table 5: Botanical classification of the plant

Kingdom	Plantea
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Asterales
Family	Asteraceae
Genus	Cynara
Species	*Cynara cardunculus*
Variety	*Cynara cardunculus var. scolymus*

2.4 Phytochemical composition of the Artichoke

The artichoke is nutrient rich vegetable and presents a complex composition (

Table 6) which varies among the different botanical parts of the plant (head, bracts, leaves, and stem) reviewed in previous studies **(Al-Subhi,2020 ; Amrani, Al Amrani, & Khalaf Aneed , 2023; Colombo et al.,2024)**. It is primarily composed of water, which represents 91% of its total weight. In 100 g of raw portion there is 3 g of protein, 0.2 g of fatty acids and 11 g of carbohydrates, of which 5.4 g is dietary fiber **(Colombo, 2024, Olas et al., 2024)**. It provides 41.04 % essential Amino Acids of the total amino acids. Artichoke is rich in vitamins, sterols, minerals, terpenoids, in smaller amounts in carotenoids and most importantly rich in polyphenols **(Lattanzio et al., 2009; Rochetti et al., 2020; Colombo et al., 2024)**.

Table 6: The biological and thrapeutic functions of *Cynara scolymus* compounds (Lambardo et al., 2018; Zayed et al., 2020 Keramati et al., 2022; Salata et al., 2022; Alicandri et al., 2023; Colombo, 2024)

Class of the bioactive compound	The bioactive elements	Biological and therapeutic role
Flavonoids	- Luteolin - Apegenin - Anthocyanins - Quercitin	- Antioxidant - Anti-inflammatory - Antimicrobial - Anticancer activities - vasorelaxant

	- Cyanidin	- Hepato-protective - Antiglycaemic - anticholesterol
Phenolic acids	**Caffeic acid derivatives** - chlorogenic acid, - cynarine - 1,5-dicaffeoylquinic acid - neochlorogenic acid	- Chemopreventive - Hepato-protective - Inhibition of mutagenic compounds formation (nitrosamines) - Prevention of atherosclerosis and other cardiovascular diseases
Terpenoids (sesquiterpene lactone STLs)	**Present mainly in the bracts:** - Cynaropicrin - Grosheimin - Isoamberobin	- Antihyperlipidemic - Antitrypanosomal - Anti-malarial - Anti-inflammatory - Anti-spasmodic - Anti-photoaging - Anti-tumoral
Dietary fibers	- Inulin	- Prebiotic Activity
Vitamins	- Vitamins (A, C, E and K) - B3 (Niacin), B6 (Pyridoxine), B7 (Biotin), B9 (Folates) - Lutein	- Supporting immune cells functions - Protection against environmental oxidative stress
Minerals	- K ,Fe and Zn the most abundant minerals, - P, Ca, Mg, Na, Z, and Cu	- Prevention against disorders such us anemia, neurological and immunological alterations.

2.5 Biological effects and health benefits of Artichoke

The therapeutic effects of artichoke result from multiple active compounds acting additively or synergistically illustrated previously in the (

Table 6), the health benefits of its natural extracts are well supported by numerous *in vivo* and *in vitro* studies **(Rondaelli et al., 2015).**

A study on LPS-stimulated human macrophages demonstrated that AE effectively reduced ROS production and inhibited the expression and the secretion of Interleukin-6 and CCL2. These findings suggest that *cynara scolymus* extract possesses both antioxidant and anti-inflammatory properties **(Carpentieri et al., 2022)**. This vegetable represents a promising

botanical approach for managing neuro-inflammation and various inflammation-related diseases, including neurodegenerative disorders. Studies have shown that artichoke leaf extract reduces microglial infiltration, TNF-α levels, and free radical production in rat models of neurodegeneration **(Porro, 2024)**.

Other studies and reviews have reported that polyphenols in artichoke possess also antibacterial, antifungal, and antiviral activities **(Chojnacka et al., 2021, Makarewicz et al., 2021, Colombo et al., 2024; Olas, 2024)**.

Flavonoids present in artichoke extracts such as cynarins, and sesquiterpenes are primarily responsible for the hepatoprotective effects against many liver diseases, by improving the liver function, owing to their potent antioxidant properties **(Ben Salem et al., 2019; Deng et al., 2022)**. Animal studies suggest that artichoke extracts may promote the regeneration of liver cells **(Ben Salem et al.,2015)**, and The presence of fibers helps to decrease triglycerides, LDL levels and prevents the accumulation of liver lipids **(Colombo et al., 2024, Heidarian et al.,2011)** .

2.6 Applications of Artichoke

Given its notable antioxidant, anti-inflammatory, and prebiotic properties, *Cynara scolymus L*. represents a valuable resource for the development of functional secondary products **(Ayuso et al., 2024)**:

1. **Cosmetology:** In a recent *in vivo* study, a cosmetic formulation with artichoke extract significantly improved skin roughness and elasticity in individuals with facial sagging compared to a placebo cream. The findings highlight the promising application of artichoke extract in cosmetology as a natural agent for preventing and alleviating signs of skin aging **(D'Antuono et al., 2018)**
2. **Food industry**
   - As antioxidant additive: Recent studies have explored the ability of antioxidant compounds in artichoke extracts to prevent the oxidation of edible fats and oils **(Claus et al., 2015).** The phenolic compounds in artichoke extracts help reduce lipid and protein oxidation, preserve the red color of meat, and prevent the formation of off-flavors and odors **(Demir & Ağaoğlu, 2021; López-Pedrouso et al.,2022)**
   - In Cheese production: Artichoke is a rich source of aspartic proteases, known as cardosins A and B, which have proven effective as milk coagulants and can substitute calf rennet without altering cheese quality **(Ayuso et al., 2024; Colombo et al., 2024)**.

A prominent example is the Italian cheese "*Caciofiore dei Sibillini*", which is well-known for using enzymatic extracts from *Cynara cardunculus L*. **(Ricceri et al,. 2016)**.

- In yogurt production: Inulin from artichoke has been shown to improve probiotic viability in yogurt, leading to faster acidity development and a shorter fermentation time. Additionally, it enhances yogurt's rheological properties by acting as a natural thickener, promoting protein aggregation through hydrogen bonding. These effects also contribute to increased viscosity and creaminess **(Ayuso et al., 2024; Colombo et al., 2024)**.

III Orange peel (citrus sinensis L.)

Sweet orange (*citrus sinensis*) is the most widely cultivated *Citrus* species. Orange fruits processing produces about 10 million MT of waste annually. In juice production the generation of peel waste is practically 50-55% of the fresh fruit mass **(Suri et al., 2022)**. The accumulation of orange peels after processing leads to environmental pollution and poses serious health risks. Nonetheless, these citrus processing wastes represent a valuable resources for the recovery of bioactive compounds **(Suri et al., 2021)** such as polyphenols and essential oils which render them suitable for diverse applications and provide broad scope for scientific research, aimed at developing novel therapeutic approaches to prevent diseases, particularly those related to inflammation.

3.1 Distribution

Citrus sinensis is cultivated across numerous tropical and subtropical regions situated roughly between 35° north and 35° south latitudes originated in Southeast Asia, now it is grown and cultivated in the worldwide lands. In 2020 the production of sweet orange accounts for about 75.5 million tons and the main producers were Brazil, India, and China **(Seminara et al., 2023)**. Additionally, *C. sinensis* is distributed in north Africa countries (**Figure 7**) including

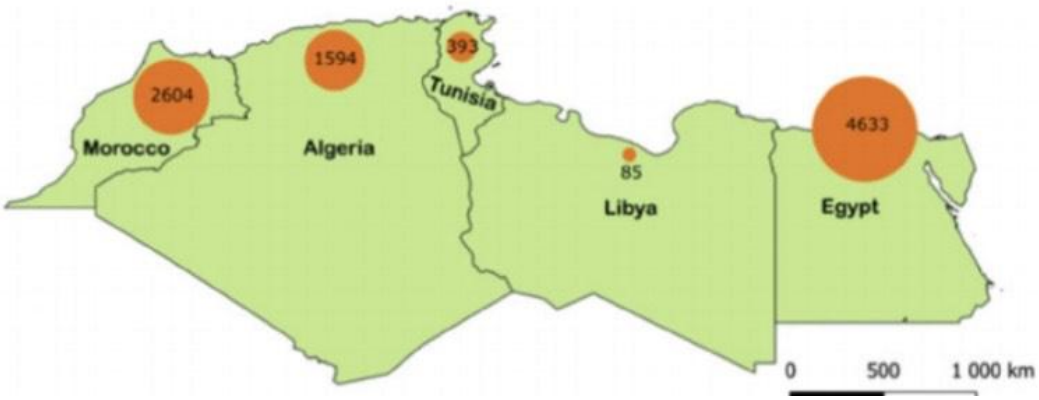

Figure 7: Citrus distribution and production in north Africa **(Kazi Tani., 2024)**

Egypt, Morocco, Tunisia, Libya and in Algeria, where it is predominantly located in the coastal plains of the central and western parts of the country **(Boudedja et al., 2024)**. According to FAO statistics Algeria ranked as the 3rd largest producer of sweet orange in the Arab Maghreb union, and 19th worldwide producing approximately 1289.9 and 1372.4 Thousands tons respectively during the years 2015 and 2016 **(Youcef-Ettoumi et al., 2021)**.

3.2 Morphological description

The *citrus sinensis* is an evergreen tree that grows to a height of 9 to 10 m with large spines on its branches. The tree is composed of ovate aromatic leaves and axillary white flowers with yellow stamens in the center. Each flower contains five petals, growing either singly or in clusters of six flowers. The fruit is round, orange to yellow in color **(Suri et al., 2022)**. Anatomically, it consists two main parts shown in (**Figure 8**). The endocarp (pulp) which is segmented into sections called carpels separated by thin epidermis containing juice sac glands called vesicles and seeds. While the pericarp (peel) consists of an outer cuticle covering the epidermis which includes the flavedo (exocarp) called zest. It is the outer colored layer composed of parenchymatous cells and contains oil glandes filled with essential oils, waxes, fatty acids, steroids, and phytochemicals such as chlorophyll, carotenoids, phenolic compounds and enzymes. The inner layer with white color and spongy texture known as the albedo (mesocarp). It is composed from joined tubular-like cells with large intracellular air spaces. This region is rich in dietary fibers, gelatin and lignin **(Favela-Hernandez et al., 2016; Saini et al., 2022)**.

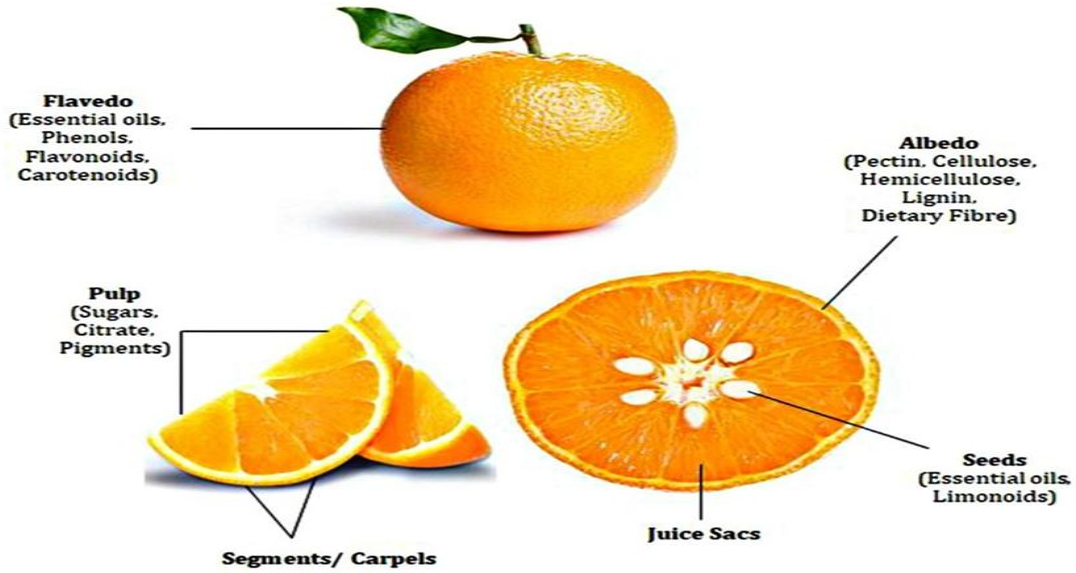

Figure 8: schematic illustration of orange fruits morphology **(Suri et al,2022)**

3.3 Botanical classification of *Citrus sinensis*

Citrus sinensis known as sweet orange is a fruit that belongs to Rutaceae family and represents the most important species in the Citrus genus **(Seminara et al., 2023)**. The botanical classification of *Citrus sinensis* is represented in (**Table 7**):

Table 7: Classification of *Citrus Sinensis* **(Chand et al., 2020)**

Kingdom	Plantae
Phylum	Spermetophyta
Subphylum	Angiospermae
Class	Dicotyledonae
Order	Rutales
Family	Rutaceae
Genus	Citrus
Species	*Citrus sinensis*

3.4 Phytochemical composition of the orange peels

Sweet orange peels contain various chemical compounds with effective biological functions illustrated in the (**Table 8**):

Table 8: The biological and therapeutic functions of *Citrus sinensis* peel compounds (Russo et al., 2021)

Class of the bioactive compound	**Bioactive Elements**	**Biological and therapeutic role**
Essential oils	Limonene (98.238%), sabinene, β-myrcene, β-pinene, α-pinene	Antioxidant Anti-inflammatory Antimicrobial **(Anwar et al,2023)** Anti-cancer **(Homburger, et all ,1971)**
Flavonoids	Narirutin, Hesperidin Nobiletin, Tangeretin, Sinensetin (PMFs)	Anti-inflammatory Antioxidant Anti-cancer **(Tripoli et al.,2007)**
Sterols	β-sitosterol	Anti-inflammatory **(Sun,et all. 2020)**. Hypocholesterolemic **(Sugano, et all ;1977)**
Vitamins	Ascorbic acid (Vitamin C)	Anti-inflammatory Skin health and collagen synthesis activation **(Michels, 2011).**

Terpenoids	Limonoids	Antioxidant, anticancer, antiviral, cholesterol-lowering, liver-protective

3.5 Biological effects and health benefits

Sweet orange peels are rich in multiple bioactive compounds as represented in the previous table (**Table 8**). Citrus peel extracts were studied for their different biological and therapeutic roles mainly antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.

As regards the anti-inflammatory and antioxidant effects; flavonoids are the main phenolic compounds involved in regulating the expression of genes involved in the inflammatory response such as COX-2 , TNF-alpha , NFkB, IL-1ß, IL-6;IL-8 and ICAM-1 **(Gosslau et al., 2014; Osarumwense, 2017)**. Therefore, flavonoids rich extracts from orange peels demonstrated a high antioxidant activity due to their capacity in the reduction of free radicals **(Triyono et al , 2024).** In addition**,** OPEs has potent antimicrobial and antifungal activities. These effects are principally related to the presence of flavanone glycosides **(Anwar et al., 2023)**.

The anti-cancer activity of OPEs concerns the inhibition of cancer cells growth by the cytotoxic effects of their essential oils containing decanal, linalool, valencene, and octanal. An *in vitro* study on Hela cells (human cervical epithelial cells from cervical cancer) has confirmed the anti-proliferative effect of the orange peels **(Triyono et al, 2024).**

Other investigations were also performed on the neuroprotective potential of orange peel extracts. It was demonstrated that OPEs has the ability to reduce oxidative stress in the brain, suggesting a role in preventing the progression of Alzheimer's disease. **(Chakraborty et al., 2024).**

OPEs were studied for their potential effects on hypercholestoremia and hyperglycemia. Flavonoids like hesperidin and naringin can suppress alpha-amylase enzyme, which is responsible for converting complex carbohydrate into glucose, and stimulating insulin secretion. Their role in decreasing cholesterol and triglyceride levels refers to their capacity in inhibiting both HMG-CoA reductase and acetyl-coenzyme A acetyltransferase **(Muhtadi et al., 2015).**

3.6 Applications of sweet orange peels

Sweet orange peels are valued for their organoleptic qualities and significant nutraceutical properties which make them suitable for numerous applications across various industries **(Revathi et al., 2025)**:

1. Culinary Uses: As flavoring agent, the zest or peel of sweet orange is commonly used to enhance the flavor of desserts, beverages, and various dishes. The essential oils extracted from the peel, particularly limonene, provide a citrusy aroma and flavor **(Wedamulla et al. 2022)**.
2. Cosmetic Uses: Due to its high vitamin C and antioxidant content, orange peel is often used in skincare products to improve skin health, reduce wrinkles, and provide a glowing effect. It is also used in facial masks and scrubs **(Jaiswal & Gaur., 2023)**
3. Medicinal Uses: Sweet orange peel is used in traditional medicine to promote digestion and relieve symptoms such as indigestion and bloating. It is believed to have carminative properties **(Adebiyi & Raji., 2015).**
4. Industrial Uses: Orange peel oil, primarily composed of limonene, is used in eco-friendly cleaning products due to its grease-dissolving and antimicrobial properties **(Balarezo Saltos et al., 2024)**

Chapter 03:
Materials and methods

IV Materials

4.1 Animal materials

Our study was conducted on (150-200g) male *Wistar albino* rats purchased from Pasteur Institute, Algiers, Algeria. The animals were kept in cages with open access to water and food with 12/12h light/dark cycle and controlled room temperature.

4.2 Vegetal materials

In this study, we selected artichoke bracts and sweet orange peels as the by-products for investigation. Fresh artichoke vegetables and oranges were obtained, and their respective by-products were collected immediately prior to extracts preparation.

4.2.1 Preparation of the hydro-alcoholic extract of artichoke bracts (*Cynara scolymus*)

We used purple artichokes obtained from the market; the extraction was carried out according to **(EL-Hadidy et al., 2022**) protocol:

1. The artichoke were cleaned with water, the bracts were collected and then dried at 40℃.
2. A total of 10 g of the dried bracts were suspended in 100 ml of an Ethanol-Water solution (70:30) and incubated at 4℃ for 48h.
3. After incubation the suspension was centrifuged at 4000 rpm for 10 min.
4. The resulting supernatant was collected and the Ethanol was removed by vacuum evaporation at 40℃ using a Speed Vac.

4.2.2 Preparation of the aqueous extract of the orange peel (*Citrus sinensis*)

We used untreated oranges harvested from a garden. The extraction was carried out based on the method outlined by **(Gotmare & Gade., 2018)**, incorporating certain modifications:

1. We washed and dried the oranges.
2. Using a peeler, we collected the orange peel.
3. Then the peels were dried at 40℃ and ground into a finer powder.
4. A total of 10 g of the powder was mixed with 100 ml of boiling distilled water and stirred for 30 min.
5. After centrifugation at 8000 rpm for 10 min, the supernatants were lyophilized and stored at -20℃.

4.3 Laboratory equipment

The experimental part was conducted with the following materials and laboratory apparatus (**Figure 9**):

Figure 9: Presentative photographs of key materials and equipment used in the experiment

4.4 Solutions

The working solutions employed in this study represented in (were prepared as follow:

- λ-carrageenan (1%) was prepared in (0,9 %) NaCl physiological saline.
- Turk solution was prepared by mixing 1 ml gentian violet with 1 ml of glacial acetic acid. Then completing the volume to 100 ml with distillated water. It was used for neutrophils staining.
- Physiological saline (0.9%) NaCl was used for peritoneal lavage.

V Methods

5.1 *In vivo* anti-inflammatory activity tests of ABE and OPE

In this work, we performed xylene- induced ear edema, carrageenan-induced paw edema and peritonitis tests to evaluate the anti-inflammatory activity of the tested extracts. These experiment models are well validated for studying acute inflammation.

In this study, the animals were randomly divided into 5 groups of 6 animals each:

- **Positive control group:** received no substance or treatment.
- **Negative control group:** received an injection of 0.2 ml of physiological saline (0.9% NaCl) without any additional treatment.
- **Reference group:** received 1 ml of aspirin at a dose of (400 mg/Kg) orally.
- **Test group (1) and (2):** Received 1ml of artichoke extract (ABE) at a dose of (500mg/Kg) by gavage one hour before any induction of inflammation.
- **Test group (2):** Received 1ml of orange peel extract (OPE) at a dose of (500mg/Kg).

5.1.1 Xylene-induced ear edema test in rats

This model of experiment were performed according to **(Sun et al., 2018)** protocol. A (60µl) of xylene solution were applied in the anterior and posterior surfaces of the right ear of each animal. And the left ear was considered as control. The thickness of both ears was measured before xylene application at (t0) and at (t1, t2, t3 and t4) each hour using a digital caliper. Finally the percentage of edema inhibition was calculated according the following equation:

$$\textbf{Inhibition (\%)} = [1 - \frac{ET}{EC}] \times 100 \qquad (1)$$

Where:

Et: thickness of right ear -thickness of the left ear (Test groups).

Ec: thickness of right ear -thickness of the left ear of the Positive control group.

5.1.2 Carrageenan-induced paw edema test in rats

The carrageenan induced paw edema test was performed according to **(Oluwatoyin et al., 2019)** protocol. Where we injected the right paw hind of pre-treated rats with (100µl) of (1%) carrageenan solution. Using the digital caliper, the thickness of the paw edema was measured at the following times (t0, t1, t2, t3 and t4) each hour. The percentage of edema inhibition was calculated using the precedent equation **(1).**

5.1.3 Carrageenan-induced peritonitis in rats

This inflammatory model was performed according to **(Radulović et al. 2024).** One hour post-treatment (AE, OPE or Aspirin). Then peritonitis was induced in rats by an intraperitoneal injection of (200µl) of (1%) carrageenan solution as represented in (**Figure 10**). Four hours following the induction of inflammation, euthanasia was performed using chloroform asphyxiation.

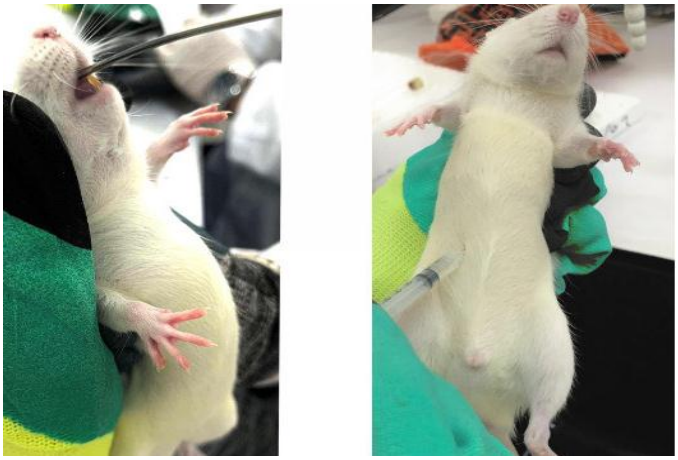

Figure 10: Realistic representative photographs (2025): Oral administration (left). Intraperitoneal injection of carrageenan or (NaCl) solution (right).

We washed the peritoneal cavity after the sacrifice of animals with 3 ml of physiological saline as shown in (**Figure 11**).

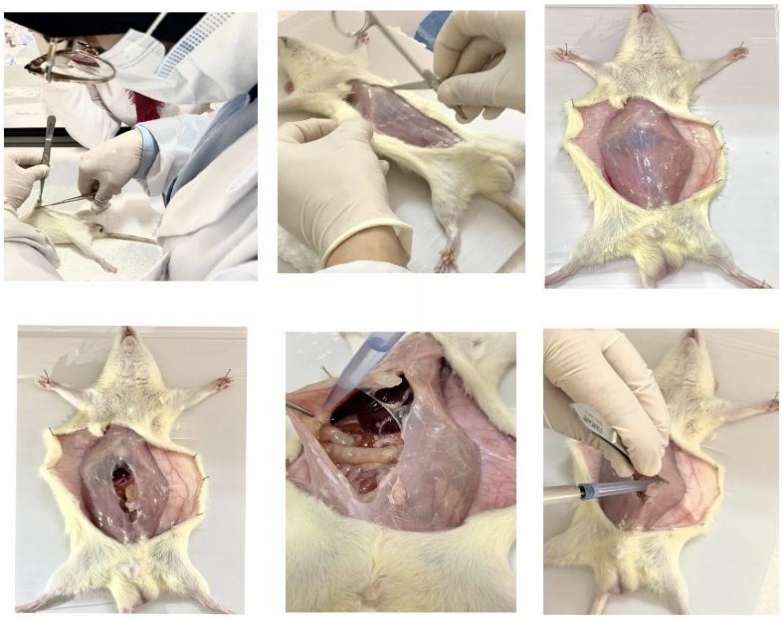

Figure 11: Rats dissection

1. Neutrophils counting

Neutrophils count were performed using a Hemocytometer Malassez by light microscopy with ×10 objective (**Figure 12**).

The cellular concentration **[C]** were expressed by (10^6 cells) and calculated as following:

$$[C] = N \times F \times 1000 \times V \quad (2)$$

Where:

N: neutrophils number counted in the malassez surface (cells/ml); **F**: factor of dilution 100 ml; **V**: volume of collected exudate sample).

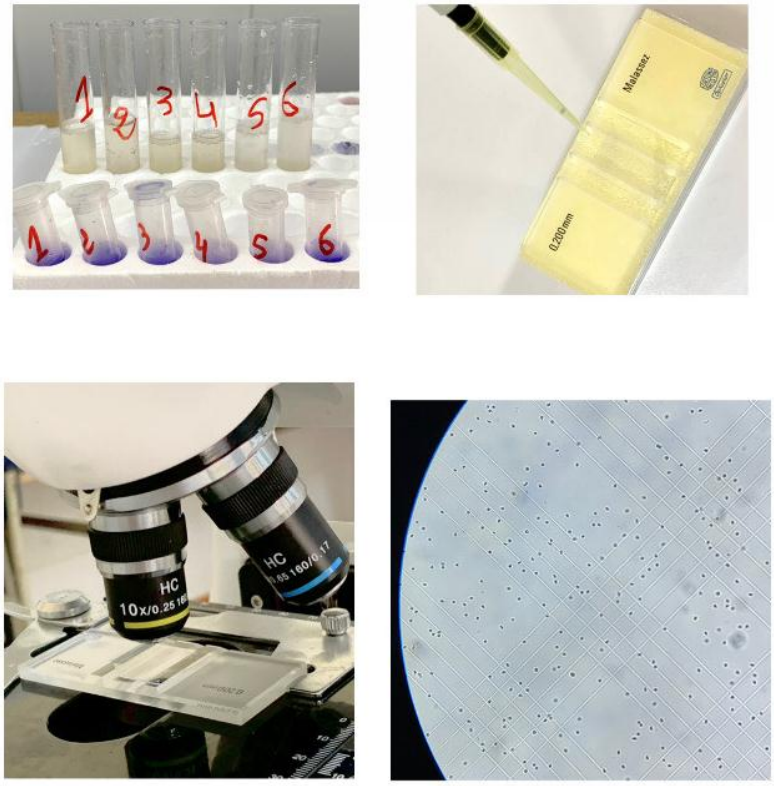

Figure 12: Montage of real images illustrating: key steps from samples preparation to microscopic counting

2. Percentage of neutrophils migration inhibition (%)

The level of inhibition of PMNs migration in the peritoneal cavity by **AE** and **OPE** extracts was calculated according to the following equation:

$$\textbf{Inhibition (\%)} = \left[1 - \frac{NT}{NC}\right] \times 100$$

Where:

(**NT:** neutrophils number of the test groups**, NC:** neutrophils number of the positive control group).

5.2 Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was conducted using Student's paired t-test in Microsoft Excel, which was also used to generate the charts. Differences were considered statistically significant at a value of ($P \leq 0.05$).

Chapter 04:
Results & Discussion

VI Results

In this research, the extract of artichoke bracts and sweet orange peels were obtained by hydro-alcoholic and aqueous extraction respectively, where the evaluation of their anti-inflammatory activity was performed on *Wister albino* rats. Using ear edema, paw edema and peritonitis as acute inflammation models for evaluating different phases and mechanisms of inflammation including edema formation and leucocytes migration. This section provides a comprehensive analysis and discussion of the following obtained results.

6.1 Evaluation of the anti-inflammatory effect of artichoke bracts extract (ABE)

Based on the chemical composition of artichoke bracts as reported in the literature, we investigated the anti-edematous and leucocytes migration inhibitory effects of the extract using the mentioned *in vivo* models. By monitoring the increase or decrease in edema thickness and neutrophils number, we assessed the anti-inflammatory effect of the extract.

6.1.1 Evaluation of edema inhibition by artichoke bracts extract (ABE)

The anti-inflammatory potential of artichoke bracts extract (ABE) was first evaluated using the xylene-induced ear edema model in rats. Topical application of (60 µl) xylene on the ear significantly increased ear thickness, indicating acute inflammation. As shown in the data (**Figure 13**), the edema thickness reached its maximum progression within the first hour, measuring 0.19 mm ±0.033.

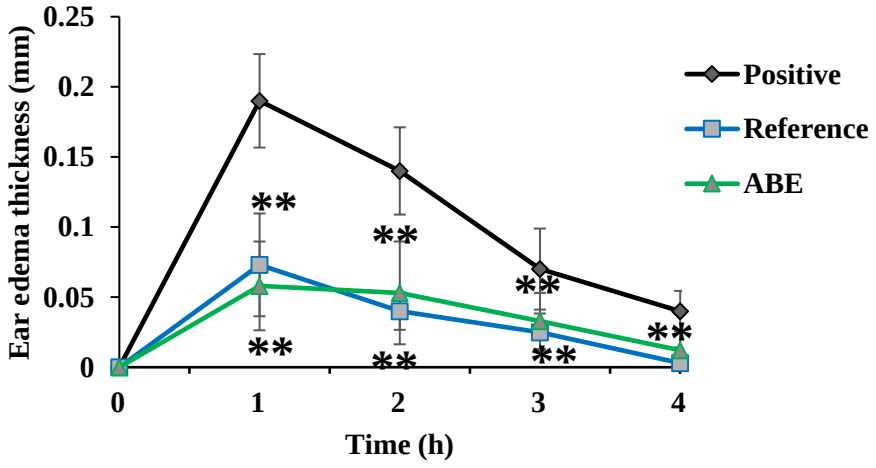

Figure 13: Effect of artichoke bracts extract (500 mg/kg) and aspirin (400mg/kg)) on xylene induced ear edema thickness compared to positive control over time. The results presented as mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using student test (*t*-test)

In contrast, one hour after treatment with the extract of artichoke bracts at a dose of (500 mg/kg), the edema thickness significantly ($P < 0.01$), decreased to 0.058±0.032, indicating the anti-inflammatory effect of ABE, which inhibited edema formation by (71.8%). Oral administration of ABE also reduced ear edema compared to the reference group. The maximal inhibitory effect was observed at the third hour post-xylene injection, with ABE achieving (79%) inhibition of ear swelling, closely approaching the (84.2%) inhibition observed with the reference drug aspirin at dose of (400) mg/kg. The results of ear edema inhibition are shown in (**Figure 14**).

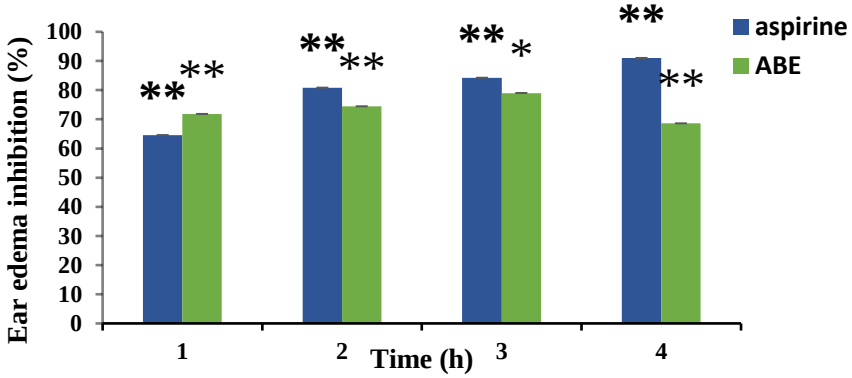

Figure 14: Inhibition percentage of ear edema induced by xylene by ABE and aspirin. Data is presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using *t*-test.

The second experimental model to study edema inhibition by ABE was the carrageenan-induced paw edema. In this test, paw edema thickness increased over four hours following injection of (100 µl) of (1%) carrageenan solution. Pre-treatment of rats with artichoke bracts extract at (500 mg/kg) and aspirin (400mg/kg) significantly ($p<0.01$) decreased paw edema thickness compared to the positive group from 3.3mm±0.4mm to 1.2mm±0.2mm at the fourth hour. But the reduction on paw edema thickness was more pronounced in the ABE group, than the reference group which reached 1.8±0.3mm (**Figure 15**). .

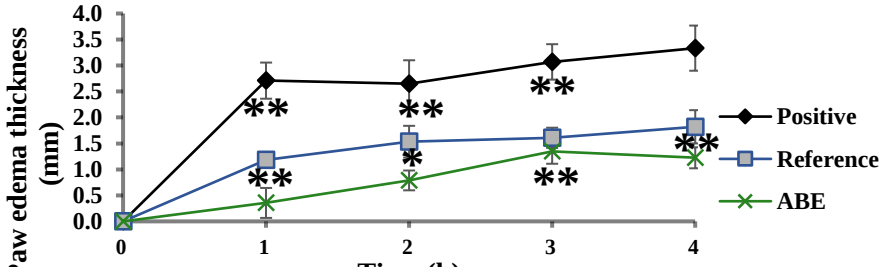

Figure 15: Effect of ABE and reference drug on carrageenan-induced paw edema thickness compared to positive control over time. The results presented as mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using student test (*t*-test).

Notably, a high inhibition percentage of paw swelling was observed in the first hour achieving (87%). The results represented in (**Figure 16**) showed no significant difference between the effects of ABE and the reference drug on paw edema inhibition, suggesting that ABE acts quickly to reduce paw edema progression.

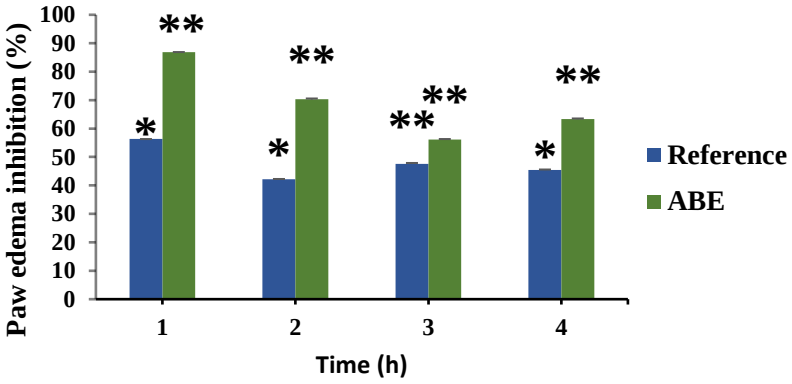

Figure 16: Inhibition percentage of (1%) λ-carrageenan induced paw edema by the reference aspirin (400 mg/kg) and ABE (500 mg/kg). Data are presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using *t*-test.

6.1.2 Evaluation of neutrophils migration inhibition by artichoke bracts extract (ABE)

To evaluate the artichoke bracts extract on neutrophil migration, the carrageenan-induced peritonitis model was employed, by an intraperitoneal injection of (200µl) of (1%) carrageenan solution. The negative control group received (200µl) of physiological saline solution using the same injection method. Four hours after peritonitis induction, this group presented a low number of neutrophils in peritoneal cavity with an average of 8.87 x 10^6 ±4.30 indicating a minimal inflammation. However, a clear inflammatory response was observed in the positive control group, with the neutrophil count reaching 295.2 x 10^6 ± 40.32 (**Figure 17**). Pre-treated animals with artichoke bracts extract at dose of (500mg/kg) by oral administration, showed a significant ($p < 0.01$) reduction in neutrophil counts which declined to 37.80 x 10^6 ±6.96 corresponding to an inhibition percentage of 87, 20%. Compared to the results observed in the reference group pre-treated with aspirin at dose of (400mg/kg) which showed a mean neutrophil number of 41.99 x 10^6 ±10.41, ABE exhibited a stronger inhibitory effect on neutrophil migration.

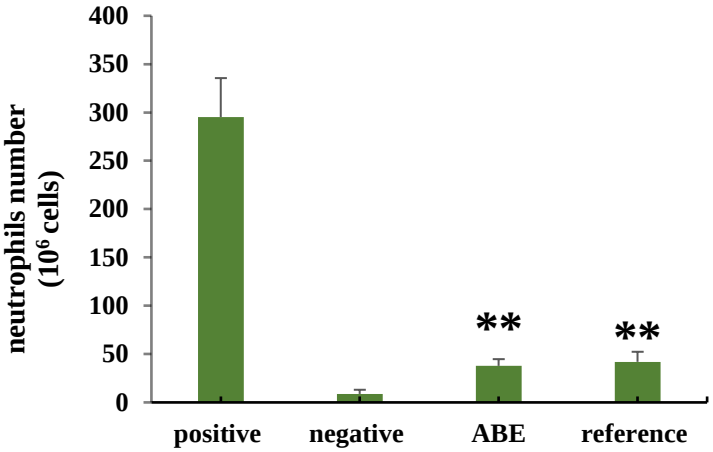

Figure 17: Evaluation of neutrophils number in (1%) λ-carrageenan-induced peritonitis of untreated group (positive) compared to negative and treated groups with (500 mg/kg) of OPE and (400 mg/kg) aspirin both were administrated orally one hour before peritonitis induction. The results presented as mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using *t*-test.

6.2 Evaluation of the anti-inflammatory effect of orange peels extract (OPE)

To evaluate the anti-inflammatory potential of orange peel extract, we investigate two models: xylene-induced ear edema and carrageenan-induced paw edema using the same reference, positive and negative control groups as previously with artichoke bracts extract.

6.2.1 Evaluation of edema inhibition by orange peels extract (OPE)

The oral administration of orange peels extract (500 mg/Kg) one hour prior ear edema induction with xylene reduced significantly (p<0.05) the ear edema. Edema thickness reduction was observed from the first hour of measurement, from 0.19 ±0.03mm to 0.062±0.03mm, and completed to decrease over time of experiment until it reached 0.020 mm ± 0.013 in the last hour (**Figure 18**).

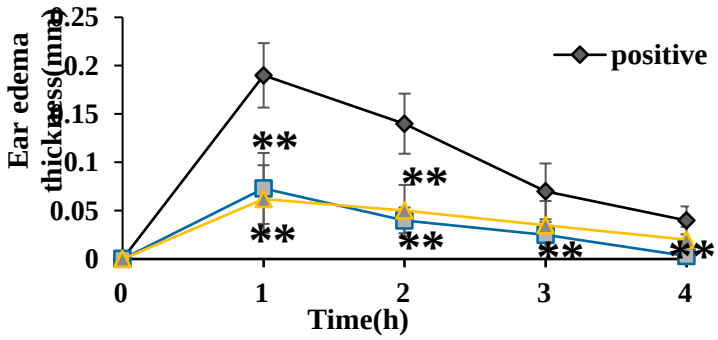

Figure 18: Effect of orange peel extract (500 mg/kg) and aspirin (400 mg/kg) on xylene-induced ear edema thickness compared to positive control over time. The results presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using *t*-test.

This aqueous extract was also able to reduce xylene-induced ear edema compared to the reference group of rats that received aspirin at dose of (400mg/kg). Aspirin reduced edema thickness until the last hour to 0.073±0.03mm. The maximum inhibition percentage was observed during the third hour of measurement reaching 77.9% and 84.2% for OPE and aspirin respectively (**Figure 19**).

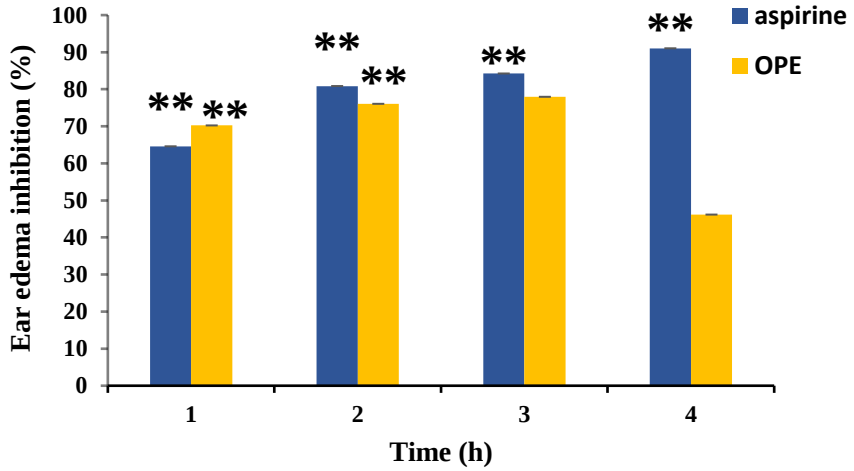

Figure 19: Inhibition percentage of xylene-induced ear edema by aspirin the reference drug (400 mg/kg) and orange peel extract (500 mg/kg). The results presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using student test.

The carrageenan-induced paw edema test was also performed to validate the inflammatory potential of OPE. After injection of (100µl) of (1%) carrageenan solution, paw edema progressively increased over a four hour observation period (**Figure 20**). However rats treated with mentioned extract at the same previously used dose of (500mg/kg) the paw edema was significantly reduced ($p < 0.01$). Compared to the positive group, paw edema thickness decreased from a maximal value 3.3 mm ± 0.4mm to a minimum of 1.3 mm ± 0.3mm, corresponding to an inhibition percentage of (72%) at (t4).

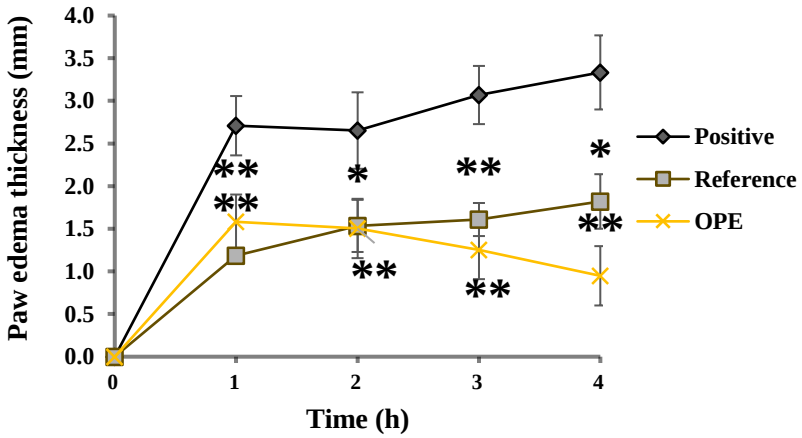

Figure 20: Effect of orange peel extract and aspirin at doses (500 mg/kg) and (400 mg/kg) respectively on (1%) λ-carrageenan-induced paw edema thickness compared to positive control over time. The results presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using Student test (*t*- test).

The pre-treatment of animals with (400mg/kg) of aspirin prior to carrageenan injection, reduced paw edema thickness over the four hour measurement period by 56%, 42%, 48% and 45% respectively. This demonstrates aspirin's immediate anti-edematous effect from the first hour. In contrast, OPE inhibited edema progression gradually, with inhibition percentages of 42%, 43%, 59% and 72% at the corresponding time points. The data shown in (**Figure 21**) illustrate the time-dependent, comparable anti-edematous effect of OPE the reference drug.

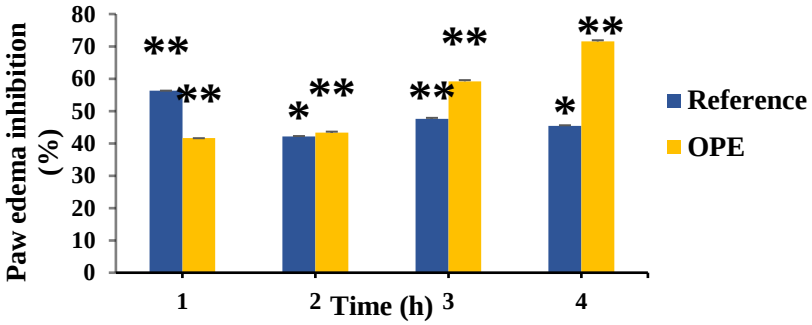

Figure 21: Inhibition percentage of (1%) λ carrageenan-induced paw edema by the reference drug aspirin (400 mg/kg) and orange peel extract (500 mg/kg). The results presented as the mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using Student test (t-test).

6.2.2 Evaluation of neutrophils migration inhibition by orange peels extract (OPE)

The effect of orange peel extract on neutrophil migration was evaluated using carrageenan-induced peritonitis model, by an intraperitoneal injection of (200µl) of (1%) carrageenan solution. The negative control group received (200µl) of physiological saline solution presented a low number of neutrophils with an average of 8.87 x 10^6 ±4.30. While in the positive control group the neutrophil count reached 295.2 x 10^6 ± 40.32. Oral administration of orange peel extract with a dose of (500mg/kg) in rats led to a significant ($p < 0.01$) decrease in neutrophil recruitment with an average value of 39.62 x 10^6 ±15.45 and an inhibition rate of 86, 58%, which was greater than that observed in the aspirin-treated group, results are shown in (**Figure 22**).

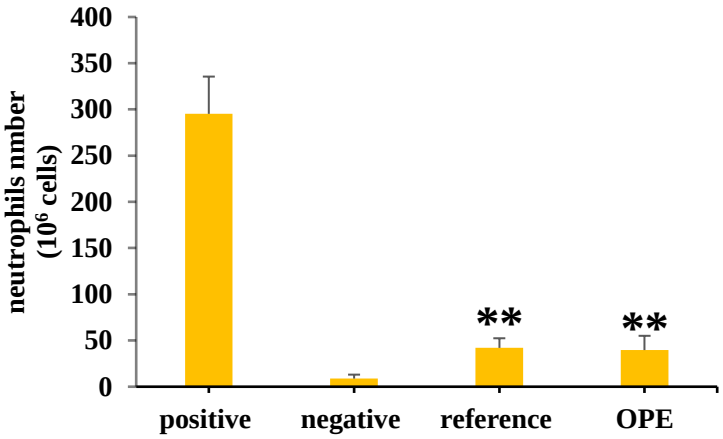

Figure 22: Evaluation of neutrophils number in (1%) λ-carrageenan-induced peritonitis of untreated group (positive) compared to negative and treated groups with (500 mg/kg) of OPE and (400 mg/kg) aspirin both were administrated orally one hour before peritonitis induction. The results presented as mean ± SEM for n=6, with significance levels (P<0.05*), (P<0.01**) using *t*-test.

VII Discussion

This study evaluated the anti-inflammatory effects of artichoke bracts extract (ABE) and orange peels extract using *in vivo* acute inflammation models. By assessing edema thickness and neutrophil migration in the peritonitis model, we aimed to elucidate their effects on both the early and cellular phases of inflammation. The following discussion interprets these findings in the context of existing literature and explores the possible mechanisms underlying their anti-inflammatory activity.

7.1 Anti-inflammatory effect of artichoke bracts extract (ABE)

7.1.1 Anti-edematous effect of ABE

The present study demonstrated that artichoke bracts extract (ABE) exhibits significant anti-inflammatory activity, as evidenced by its ability to markedly inhibit edema formation in both xylene-induced ear edema and carrageenan-induced paw edema models in rats. These results highlight the potential of ABE as a natural anti-inflammatory agent, showing efficacy comparable to that of aspirin, a well-established reference drug.

As mentioned earlier in the literature review, the initial phase of acute inflammation involves vascular changes such as vasodilation and increased permeability, mediated by chemical factors like histamine, bradykinin, and prostaglandins, which lead to edema formation **(Krishnappa, 2016, Abdul Hafid & Iran, 2019)**. ABE may inhibit this vascular phase by reducing these changes, thereby potentially limiting edema and modulating the inflammatory response. Our results indicate that ABE inhibited paw edema and ear swelling, with a percentage of inhibition that approaches or slightly differs from that of aspirin in the same experimental models. The involvement of ABE in inhibiting the early phases of acute inflammation may be attributed to its high content of bioactive compounds. Polyphenols present in artichoke extracts, including bracts extract, are key compounds that have been tested for their capacity to decrease the release of pro-inflammatory mediators such as nitric oxide (NO), prostaglandins, C-reactive protein (CRP), and fibrinogen in several studies **(Colombo et al., 2024)**. Flavonoids can influence tyrosine and serine-threonine protein kinase signaling pathways. Compounds like quercetin, caffeic acid and chlorogenic acid; found mainly in the inner bracts of artichoke, which contain higher polyphenol levels, help inhibit lipid peroxidation. Similarly, anthocyanins reduce the expression of inflammatory markers such as inducible nitric oxide synthase (iNOS), COX-2, IL-1β, and IL-6, thereby reducing inflammation **(Lopez-Corona et al., 2022)**. The anti-inflammatory effects of phenolic

compounds are linked to their ability to interfere with oxidative stress signaling and suppress pro-inflammatory pathways, including blocking the secretion of pro-inflammatory cytokines.

Supporting these findings, **Borsini et al. (2021)** investigated the antioxidant properties of artichoke by-products, including bracts, and found that bracts possess high total phenolic content and strong antioxidant activity. Their study demonstrated that bioactive compounds such as caffeic acids in bracts contribute significantly to radical scavenging capacity measured by assays like DPPH, ABTS, and FRAP. These antioxidant properties likely complement the anti-inflammatory effects observed in our study, as oxidative stress is a known driver of inflammation. Thus, the potent antioxidant capacity of artichoke bracts supports their potential as natural agents for managing inflammatory conditions and related oxidative damage.

Although few *in vivo* studies have investigated the anti-inflammatory effects of artichoke bracts extracts, our results align with the established anti-inflammatory activity of artichoke leaves. **Ben Salem et al. (2017)** demonstrated that ethanol extracts of artichoke leaves, rich in phenolic compounds such as flavonoids and tannins, significantly inhibited carrageenan-induced paw edema in rats, indicating potent anti-inflammatory effects. This activity was attributed to the high antioxidant capacity and polyphenolic content of the leaf extracts, which are known to modulate inflammatory pathways. Given the similar phenolic profiles reported in artichoke bracts, including flavonoids and caffeoylquinic acids, it is plausible that the anti-inflammatory effects observed in our study are mediated by these bioactive compounds. Thus, although direct *in vivo* evidence on bracts is limited, the consistency of our findings with leaf studies supports the potential of bracts as a valuable source of anti-inflammatory agents.

In summary, the observed anti-edematous effect of Artichoke bracts extract is maybe due to polyphenol's ability to reduce oxidative stress and suppress pro-inflammatory mediators, particularly by targeting the initial stages of inflammation. This highlights their potential as natural agents for managing acute inflammation effectively.

7.1.2 Inhibitory effect of artichoke bracts extract on neutrophils migration

This model of acute inflammation aimed to evaluate the effect of the ABE on additional inflammatory parameters specifically neutrophils migration. It allows quantifying and correlating the migration of cells within the inflammatory exudate **(Cabral et al., 2016)**. Our results demonstrated that artichoke bracts extract reduced neutrophils recruitment with high inhibition percentage compared to aspirin.

As previously discussed in the context of carrageenan-induced paw edema and xylene-induced ear edema models, the anti-inflammatory effects observed are closely related to the chemical composition of the ABE. Among the bioactive constituents, phenolic compounds play a significant role; however, sesquiterpene lactones (SL) represent another major group of secondary metabolites particularly abundant in the *Asteraceae* family, which includes *Cynara scolymus* **(Salvia & Daia., 2025)**. Notably, several SLs such as costunolide, cynaropicrin, and alantolactone have been reported to exert inhibitory effects on the activation of STAT proteins through different mechanisms **(Paço et al., 2022)**. Cynaropicrin, in particular, effectively inhibits both IL-6-inducible and constitutive STAT3 activation. Importantly, STAT3 inhibition markedly decreases plasma levels of IL-6, a key cytokine in inflammation. Moreover, STAT3 signaling plays a critical role in mediating CXC chemokine production and neutrophil infiltration, processes central to the inflammatory response **(Zhang et al., 2015)**. Given that STAT3 integrates signals from granulocyte colony-stimulating factor (G-CSF) and chemokine receptors to regulate neutrophil release, chemotaxis, and migration during immune responses, it serves as a pivotal regulator of neutrophil function and inflammation **(Elsebai et al., 2016)**. Cynaropicrin also demonstrates potent suppressive effects on key inflammatory mediators such as, IL-6, NF-α, cytokine-induced neutrophil chemoattractant-1, and nitric oxide release, highlighting its therapeutic potential in both acute and chronic inflammatory diseases. Furthermore, its anti-inflammatory properties extend to the suppression of NF-κB activation and the reduction of reactive oxygen species (ROS) generation and inflammatory cytokine production **(Elsebai et al., 2016, Paço et al., 2022)**. Although cynaropicrin is present in relatively low concentrations in artichoke bracts compared to leaves **(Eljounaidi et al., 2015)**, its documented role in inhibiting neutrophil migration underscores the importance of further investigations. Characterizing the chemical components of artichoke bract extracts, particularly the presence and activity of cynaropicrin, is essential to better understand their contribution to anti-inflammatory pathways, especially those involved in neutrophil migration inhibition. Notably there is a lack of direct studies measuring neutrophils counts or migration specifically in peritonitis models treated with artichoke extracts. Therefore, further targeted research is needed to confirm and clarify the effects of artichoke bracts extracts especially from cynaropicrin-rich extracts.

7.2 Anti-inflammatory effect of orange peels extract (OPE)

7.2.1 Anti-edematous effect of OPE

In our *in vivo* tests using xylene-induced ear edema and carrageenan-induced paw edema models, the aqueous extract of these citrus by-products demonstrated strong anti-inflammatory potential at a dose of (500 mg/kg). Dose-dependent studies on *Citrus sinensis* peel extract, including **Muhtadi et al. (2015)**, reported that this dose exhibited potential biological activities in rats, supporting its safety and efficacy for in vivo experiments. At (500 mg/kg), the orange peel extract (OPE) significantly reduced edema thickness in both tests, with inhibition percentages comparable to those of the synthetic COX-1 selective NSAID aspirin, showing only a low significant difference. This highlights the potent anti-edematous effect of OPE comparable to standard medication.

The anti-inflammatory effects observed are likely due to one or more active constituents in the extract, which may inhibit edema formation by blocking the synthesis or release of inflammatory mediators, particularly cyclooxygenase (COX) products **(Malleshappa et al., 2018)**. Like artichoke bracts, Citrus sinensis peels are rich byproducts containing high levels of polyphenols. These bioactive compounds contribute to the strong antioxidant, antimicrobial, and anticancer activities reported for sweet orange peels **(Brezo-Borjan et al., 2023)**. Furthermore, flavonoids have been detected in citrus peel extracts and tested for their antioxidant and anti-inflammatory activities in recent studies including the one of **Zheng et al., (2024)**.

Polymethoxylated flavones (PMFs), a distinctive group of flavonoids found in citrus peels, exhibit potent anti-inflammatory effects primarily by inhibiting key signaling pathways such as MAPK. They reduce the production of inflammatory mediators including COX-2, iNOS, IL-1β, IL-6, TNF-α, and prostaglandin E2, and suppress transcription factors involved in inflammation. In various in vivo models, PMFs significantly decrease edema and inflammatory responses, demonstrating strong anti-edematous and anti-inflammatory potential **(Toledo et al., 2024)**. A study by **He et al. (2016)** demonstrated that nobiletin, a major PMF in citrus peels, protects against acute liver injury induced by lipopolysaccharide and D-galactosamine in mice. Treatment with nobiletin improved liver tissue damage and significantly inhibited the production of inflammatory cytokines IL-1β, IL-6, and TNF-α by blocking NF-κB activation and activating antioxidant pathways. These findings suggest that nobiletin is a key compound involved in inhibiting inflammation through modulation of multiple inflammatory mediators and signaling pathways. Additionally, hesperidin a flavanone glycoside

belonging to flavonoid family is abundant in citrus fruits including *Citrus sinensis*. This component demonstrates a strong ability to reduce edema and inflammation. Various studies have shown that hesperidin significantly inhibits paw edema induced by different inflammatory agents such as carrageenan, with effects comparable to standard anti-inflammatory drugs like indomethacin **(Man et al., 2019)**.

In summary, the diverse chemical composition of orange peel extract, particularly its flavonoid compounds including polymethoxylated flavones (PMFs) and flavanones, contributes to its potent anti-inflammatory effects. We suggest that the synergistic action of these flavonoids explains the significant inhibitory effects observed in *in vivo* edema models, with maximum inhibition percentages reaching (67%) in carrageenan-induced paw edema and (77.9%) in xylene-induced ear edema in our study.

7.2.2 Inhibitory effect of orange peels extract on neutrophils migration

In our experiment, the anti-inflammatory effect of orange peel extract (OPE) was evaluated using the carrageenan-induced peritonitis test. The results demonstrated that OPE significantly reduced neutrophil recruitment, indicating notable anti-inflammatory potential. This effect is primarily attributed to the bioactive compounds in OPE, especially polyphenols. Citrus peel polyphenols contribute substantially to the anti-inflammatory activity of OPE. These compounds modulate the secretion of pro-inflammatory cytokines such as TNF-α and IL-6, reduce myeloperoxidase (MPO) activity, a marker of neutrophil infiltration and activation. And inhibit the activation of NF-κB by downregulating NF-κB p65 expression. Since NF-κB regulates the expression of cytokines and chemokines that attract neutrophils, its inhibition leads to decreased neutrophil migration to inflamed tissues **(He et al., 2023)**.

Furthermore, hesperidin, a major flavonoid in citrus peels, has been shown to suppress carrageenan-induced pleurisy by reducing exudate volume and leukocyte migration by 48% and 34%, respectively, compared to controls **(Jagetia, 2018)**. This highlights the synergistic anti-I nflammatory effects of citrus flavonoids.Other studies have reported that orange peel essential oil contains a high level of limonene approximately 88%, which is known for broad pharmacological activities including antibacterial, anticancer, analgesic, immune-regulatory, neuroprotective, antioxidant, and anti-inflammatory effects **(Chen et al., 2024)**. Limonene significantly reduces the production of key pro-inflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO) in LPS-stimulated macrophages, both of which play crucial roles in the inflammatory cascade **(El Hachlafi et al., 2024)**. Since our study used an aqueous extract, which typically contains low amounts of essential oils like limonene due to their

hydrophobic nature, further investigations employing other extraction methods such as hydro-distillation, organic solvent extraction and novel green methods such as solvent free-microwave extraction **(Aboudaou et al., 2019)** are needed to evaluate the anti-inflammatory effects of orange peel extracts rich in essential oils. These methods could better capture the contribution of limonene and other volatile compounds to the overall anti-inflammatory activity of orange peels.

In summary, these findings support the potential use of orange peel extracts as natural therapeutic agents for managing neutrophil-driven inflammation in peritonitis and other acute inflammatory conditions.

Conclusion and perspectives

VIII Conclusion and perspectives

In today's world, where reducing food waste is an important goal, by-products are studied for their potential as natural sources of therapeutic agents. This study focused on the anti-inflammatory potential of two plants by-product, the aqueous extract of orange peel and the hydro alcoholic extract of artichoke bracts. The experiments were performed in vivo using male *Wistar albino* rats. Three inflammation models were used: xylene-induced ear edema, carrageenan-induced paw edema, and carrageenan-induced peritonitis.

Both extracts showed significant anti-inflammatory effects. They presented a high inhibition percentage, comparable to, or greater than the reference drug (aspirin) across the different tests especially in the peritonitis model, where both plants produced results comparable to the aspirin. These beneficial effects are mainly due to the rich presence of polyphenols, flavonoids, vitamins and other bioactive compounds known for their antioxidant and anti-inflammatory activities.

Artichoke bracts extract showed notable anti-inflammatory activity in our results, suggesting that this plant part may be a valuable source of natural therapeutic compounds. Traditional extraction methods often use hazardous solvents, consume high energy, cause thermal degradation, and are time-consuming and costly **(Valisakkagari et al., 2024)**. To address these issues, green or non-conventional extraction methods have been developed and are increasingly favored for their sustainability. This research provides useful information that can support future *in vivo* and *in vitro* studies on this extract using different extraction methods particularly green extraction techniques, to identify the individual active compounds and confirm their therapeutic effects.

Orange peel extract also demonstrated a high anti-inflammatory effect, particularly in the peritonitis model, which may indicate its potential for treating chronic debilitating pathologies. Its high content of polyphenols supports its therapeutic value. Further studies focusing on the albedo, the inner layer of the peel, which is rich in hemicellulose, are warranted. Hemicellulose can bind polyphenols through hydrogen bonding and other interactions, thereby reducing their extraction yield and antioxidant capacity by trapping these compounds within the plant cell wall matrix **(Phan et al., 2016)**. Because of this entrapment, the presence of hemicellulose in the albedo may limit the availability of bioactive compounds in orange peel extracts. Therefore, eliminating or modifying the albedo layer during extraction could improve polyphenol recovery and lead to new insights regarding its anti-inflammatory potential.

Based on these findings, we confirm the pharmacological potential of artichoke bracts and sweet orange peel as natural anti-inflammatory agents. In addition, they contribute to reducing environmental pollution through the valorization of plant waste. These products could serve as a safe and effective basis for treatment of chronic disorders including chronic inflammation, minimizing the side effects often associated with conventional drug.

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