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Title

**Anti-inflammatory and antioxidant effects of
Cistanche tubulosa flowers**

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Dedication

I dedicate this humble work

To my dearest parents, for their infinite patience, love, and sacrifices that made this achievement possible.

To my cherished brother and sisters, for their constant encouragement and joyful companionship.

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ملخص

تهدف هذه الدراسة الى تقييم النشاط المضاد للالتهاب و المضاد للأكسدة للمستخلص المائي للجزء الزهري لنبته *Cistanche tubulosa* . بلغ مردود المستخلص الناتج عن الغليان حوالي 26% و اظهرت التجارب الكيميائية ان المستخلص يحتوي على كمية ملحوظة من المركبات متعددة الفينول و الفلافونويدات المقدرة بـ $56,74 \pm 1,54$ ميكروغرام مكافئ حمض الغاليك\مغ مستخلص و $10,540 \pm 0,474$ ميكروغرام من مكافئ الكيرسيتين\مغ مستخلص. لدراسة النشاط المضاد للالتهاب للنبته تم اجراء اختبار استقرار الغشاء لكريات الدم الحمراء من دم شخص متبرع سليم ، اظهرت النتائج أن المستخلص يمتلك قدرة عالية على حماية غشاء الكريات الحمراء بنسبة 100 % عند تركيز 3مغ/مل متجاوزاً فعالية الدواء المرجعي Diclofenac المستعمل للمقارنة. كما اظهر المستخلص نشاطاً مضاداً للأكسدة في اختبارات DPPH و FRAP حيث امتلك قدرة فعالة على تثبيط الجذور الحرة والاختزال، وإن كانت أدنى من فعالية مضاد الأكسدة المرجعي المستعمل BHT بقيم IC_{50} تقدر بـ $135,41 \pm 0,95$ و $30,88 \pm 2,16$ مكغ/مل و EC_{50} تقدر بـ $178,86 \pm 4,1$ و $30,56 \pm 0,79$ مكغ/مل على التوالي . بالإضافة الى ذلك، فقد ثبت ان المستخلص يمتلك تأثيراً وقائياً كبيراً ضد تحلل كريات الدم الحمراء الناجم عن AAPH، مع زيادة في زمن نصف انحلال الدم (HT_{50}) بشكل مرتبط بالتركيز. بلغت قيمة HT_{50} المسجلة 68.80 ± 0.72 دقيقة و 122.30 ± 0.22 دقيقة للمستخلص و لحمض الاسكوربيك المرجعي على التوالي بتركيز 40 ميكروغرام/مل. كخلاصة، يمكن اعتبار *Cistanche tubulosa* مصدراً واعداداً لمضادات الالتهاب ومضادات الأكسدة.

الكلمات المفتاح: مضادات الالتهاب، مضادات الأكسدة، *Cistanche tubulosa*، الإجهاد التأكسدي، المركبات الفينولية.

Résumé

Cette étude vise à évaluer l'activité anti-inflammatoire et antioxydante de l'extrait aqueux de la partie florale de la plante *Cistanche tubulosa*. Le rendement de l'extrait obtenu par décoction s'est élevé à environ 26 % et les analyses chimiques ont montré que l'extrait contenait une quantité notable de composés polyphénoliques et de flavonoïdes, estimée respectivement à $56,74 \pm 1,54$ microgrammes d'équivalent acide gallique/mg d'extrait et $10,540 \pm 0,474$ microgrammes d'équivalent quercétine/mg d'extrait, respectivement. Afin d'étudier l'activité anti-inflammatoire de la plante, le test de stabilité membranaire des globules rouges provenant du sang d'un donneur sain a été réalisé, les résultats ont montré que l'extrait possède une forte capacité à protéger la membrane des globules rouges à 100 % à une concentration de 3 mg/mL, surpassant l'efficacité du médicament de référence, le diclofénac de sodium, utilisé comme anti-inflammatoire de référence. L'extrait a également montré une activité antioxydante lors des tests DPPH et FRAP, où il a démontré une capacité efficace à inhiber les radicaux libres et à réduire ces derniers, bien que cette activité soit inférieure à celle de l'antioxydant de référence utilisé, le BHT, avec des valeurs de IC_{50} $135,41 \pm 0,95$ et $30,88 \pm 2,16$ µg/mL et des valeurs EC_{50} $178,86 \pm 4,17$ et $30,56 \pm 0,79$ µg/mL respectivement. De plus, l'extrait s'est révélé posséder un effet protecteur important contre l'hémolyse induite par l'AAPH, avec une augmentation du temps de demi-vie hémolytique (HT_{50}) proportionnelle à la concentration. Les valeurs de HT_{50} enregistrées étaient respectivement de $68,80 \pm 0,72$ minutes et $122,30 \pm 0,22$ minutes pour l'extrait et l'acide ascorbique de référence, à une concentration de 40 microgrammes/ml. En conclusion, *Cistanche tubulosa* peut être considérée comme une source prometteuse d'agents anti-inflammatoires et antioxydants.

Mots-clés : Anti-inflammatoires, antioxydants, *Cistanche tubulosa*, stress oxydatif, composés phénoliques.

Abstract

This study aims to evaluate the anti-inflammatory and antioxidant activity of the aqueous extract of the flowering parts of the plant *Cistanche tubulosa*. The extraction yield from decoction was approximately 26%, and chemical analyses showed that the extract contains a significant amount of polyphenolic compounds and flavonoids, estimated at 56.74 ± 1.54 micrograms of gallic acid equivalent / mg extract and $10,540 \pm 0.474$ micrograms of quercetin equivalent/mg extract, respectively. To study the plant's anti-inflammatory activity, a red blood cell membrane stability test was conducted using blood from a healthy human donor, The results showed that the extract possessed a high ability to protect red blood cell membranes, with 100% protection at a concentration of 3 mg/mL, surpassing the efficacy of the reference drug diclofenac used as anti-inflammatory standard. The extract also exhibited antioxidant activity in DPPH and FRAP assays, demonstrating an effective ability to inhibit free radicals and reduce oxidized compounds, although its efficacy was lower than that of the reference antioxidant BHT, with IC_{50} 135.41 ± 0.95 and 30.88 ± 2.16 mg/mL and EC_{50} 178.86 ± 4.17 and 30.56 ± 0.79 mg/mL, respectively. In addition, the extract was found to have a significant protective effect against AAPH-induced hemolysis, with a concentration-dependent increase in the half-life of hemolysis (HT_{50}). The recorded HT_{50} values were 68.80 ± 0.72 minutes and 122.30 ± 0.22 minutes for the extract and the ascorbic acid reference, respectively, at a concentration of 40 μ g/mL. In conclusion, *Cistanche tubulosa* can be considered a promising source of anti-inflammatory and antioxidant compounds.

Keywords: anti-inflammatory agents, antioxidants, *Cistanche tubulosa*, oxidative stress, phenolic compounds.

List of abbreviations

AAPH: 2,2'-Azobis (2-methylpropionamidine) dihydrochloride

AP-1: Activator Protein-1

BHT: 2,6-Di-tert-butyl-4-methylphenol

CAT: catalase

COX: The enzyme cyclooxygenase

CYT P450: Cytochrome P450

DPPH: 1,1-diphenyl-2-picryl-hydrazyl

EC₅₀ : Effective concentration 50%

Fe2-TPTZ: ferrous-tripyridyltriazine

Fe3-TPTZ: ferric-tripyridyltriazine

FRAP: Ferric reducing antioxidant power

GPx: glutathione peroxidase

GSH: glutathione

GSSG: Glutathion disulfide

HT₅₀: Temps de demi-Hémolyse

IC₅₀: Concentration inhibitrice à 50%

IL: interleukin

LDL: low density lipoprotein

NF-κB: kappa-light-chain-enhancer of activated B cells

NO•: Nitric oxide

NO₂•: Nitrogen dioxide radical

Nrf2: Nuclear factor erythroid 2-related factor 2

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

ONOO-: peroxynitrite

PBS solution: Phosphate-Buffered Saline

PMNs: Polymorphonuclear leukocytes

PUFA phospholipids: polyunsaturated fatty acids

RAGE: Receptor for AGEs

SAIDs: Steroidal Anti-Inflammatory Drugs

SOD: superoxide dismutase

TCA: trichloroacetic acid

TNF-α: Tumor Necrosis Factor alpha

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INTRODUCTION

Introduction

Keeping in mind the role of inflammation in the pathophysiology of numerous diseases, we examine the processes that initiate inflammation and the therapeutic options (Baljit Kaur & Palwinder Singh, 2022). An imbalance between the generation of free radicals and reactive metabolites, also known as oxidants or reactive oxygen species (ROS), and their removal by defense mechanisms, also known as antioxidants, is known as oxidative stress. Important biomolecules and cells are harmed by this imbalance, which may have an effect on the entire.

The intricate connection between oxidative stress, inflammation, and the emergence of chronic illnesses has drawn more attention from scientists in recent decades. Numerous clinical disorders, including as cardiovascular disease, renal disease, diabetes, neurological diseases, and even cancer, have been linked to oxidative stress and chronic inflammation (Altanam *et al.*, 2025). Even while synthetic medications, such as SAIDs and NSAIDs, are the most widely used and successful anti-inflammatory agents, they typically require long-term therapy and have a negative effect on human health. Many attempts have been made to investigate the effectiveness of medicinal plant extracts and their bioactive ingredients in order to get around the drawbacks of traditional synthetic medications (Gandhi *et al.*, 2022). Significant pharmacological activity, including anti-inflammatory activity, is demonstrated by medicinal plant extracts, either as a result of the combined action of several powerful phytochemicals or the strong activity of individual bioactive components (Gandhi *et al.*, 2022).

The present study is situated within the broader scientific context of oxidative stress and inflammation, two interconnected processes that underlie numerous chronic diseases. The primary objective of this study is to investigate the anti-inflammatory and antioxidant activity of an aqueous extract from the flowering parts of *Cistanche tubulosa*, a plant used in traditional Algerian medicine for its antioxidant and immunomodulatory effects.

This work's objectives are as follows:

- Preparation an aqueous extract from *Cistanche tubulosa*'s flowers.
- Analysis of the extract's polyphenols and flavonoid content.
- Investigation of the extract's anti-inflammatory properties using the membrane stability hemolysis test, an *in vitro* experimental paradigm.
- The antioxidant activity of the extract under study was assessed using the AAPH, DPPH, and reducing power tests.

Bibliographic part

Chapter I: inflammation and stress oxidative overview

Inflammation and stress oxidative overview

I. Inflammation

Inflammation, which manifests physically as heat, swelling, redness, and pain, is the body's non-specific first-line physiological reaction to damaging stimuli such as pathogens, damaged cells, toxins, or radiation. In essence, it is the body's defense mechanism that involves the activation, recruitment, and action of innate and adaptive immunity cells to eliminate harmful stimuli. The affected tissues are then restored to their normal structural and functional state by starting the processes of tissue repair, regeneration, and remodeling. No doubt controlled inflammation is beneficial, but if it persists, it can lead to cancer, atherosclerosis, rheumatoid arthritis, obesity, Chronic Obstructive Pulmonary Disease, diabetes, asthma, Inflammatory Bowel Disease, multiple sclerosis, and neurodegenerative diseases like Alzheimer's (B. Kaur & P. Singh, 2022).

I.1. Acute inflammation

The celsian tetrad of dolor, calor, rubor, and tumor, as well as the loss of tissue and organ function in severe cases, result from the acute inflammatory response, which is characterized by an influx of cytolytically activated innate immune system cells and the release of inflammatory mediators (**Table 1**) that increase vascular relaxation, vascular permeability, and the extravasation of large molecules from the circulation to the site of tissue damage (Oronsky *et al.*, 2022).

Table 1. Summary of inflammatory mediators and their functions (Ali *et al.*, 2023).

Mediators	Functions
IL-1	Macrophages activation and T-cell activation, fever, hypotension
IL-6	Acute-phase protein production, Naïve B-cell and T-cell proliferation
IL-8	Chemotactic signals for T-cells and neutrophils
TNF- α	Activation of PMN and endothelial cells, shock-like syndrome
PAF	Activation of endothelial cells and platelets, histamine release from platelets
NO	Smooth muscle relaxation, generation of cytotoxic peroxynitrite
PGE ₂ and PGI ₂	Vasodilatation
TXA ₂	Increased pulmonary resistance
LTC ₄ , LTD ₄ , LTE ₄	Increased pulmonary capillary permeability

I.1.1. Vascular phase

The reaction is quick, happening within a minute, and is characterized by changes in local microcirculation. The extravasation of cells and plasma proteins into the tissues is made possible by the release of mediators, which causes dilatation of blood vessels with an increase in local blood flow, a shift in vascular permeability, and an increase in intercellular windows. Heat and redness are partially explained by increased microcirculatory flow. Plasma exudation increases pressure on nearby nerve endings and produces tissue distension, which results in edema. This clarifies the pain and swelling symptoms (Batteux & Weill, 2003).

I.1.2. Cellular phase

Leukocyte inflow into the extravascular interstitial space characterizes the cellular phase, which comes after the vascular phase. It takes place in three stages. Innate immune cells, mainly neutrophils and monocytes/macrophages, are mobilized during the first stage and act within minutes to eradicate infections and injured tissue. An early non-adaptive response is thus set off, involving cells with comparatively stable receptors that aid in the elimination of microbes. Lastly, the body initiates an adaptive immune response based on the specialized activation of B and T lymphocytes if these early mechanisms are not enough to control the infection. This ensures the pathogen's final eradication and the development of immunological memory (Batteux & Weill, 2003).

I.1.3. Resolution phase

The extent of tissue damage determines the resolution, or repair, phase. In fact, under ideal circumstances, neutrophils remove the harmful substances while macrophages phagocytose the breakdown products and cellular debris (Batteux & Weill, 2003). The typical acute-phase response, which is marked by a rapid infiltration of PMN leukocytes and a local production of prostaglandins and leukotrienes due to the participation of polymorphonuclear leukocytes (PMNs), occurs at the outset of inflammation with the release of TNF- α . Then, the "class switch" takes place as a result of eicosanoids involvement. This suggests that the profile of eicosanoid lipid mediators changes to "reprogram" PMN leukocytes in the exudate to support the resolution phase as exudate generation alters during the acute phase. The type of the eicosanoids varies as the exudate's composition shifts, and the lipid mediators produced and discharged into this environment alter with time (Calvino, 2021).

I.2. Chronic inflammation

Chronic inflammation can lead to a number of secondary biological reactions that are linked to an increased risk of chronic illnesses and disorders. When inflammatory reactions occur without a real stimulation, chronic inflammation in tissue typically results. It typically arises from infections that are not eliminated by host defense mechanisms or endogenous defense mechanisms. They can also result from unbreakable chemical or physical substances, as well as from genetic susceptibilities. Important causes of chronic inflammation include persistent foreign bodies, ongoing chemical exposures, recurring acute inflammation, or certain infections. Depending on the kind of inflamed cells and organ, chronic inflammation can have a variety of molecular and cellular processes (Arulselvan *et al.*, 2016).

I.3. Anti-inflammatory

I.3.1. Non-steroidal anti-inflammatory

Among therapeutic medicines, nonsteroidal anti-inflammatory medications (NSAIDs) are the most widely used class. The enzyme cyclooxygenase (COX), which is in charge of converting arachidonic acid into prostaglandins, is inhibited by NSAIDs which are a class of drugs that are primarily used to treat fever, pain, and other types of inflammation. It is important to note that COX-1 and COX-2 are the two primary COX enzymes with distinct roles. In fact, COX-1 is constitutively expressed and contributes to the preservation of homeostasis in the kidneys, gastrointestinal tract, and other organs. However, COX-2 is constitutively produced in several tissues and plays a part in some physiological processes, including kidney function and healing, even though it is mostly induced during inflammation (Khalil *et al.*, 2024). Its inactivation by NSAIDs leads to the inhibition of the synthesis of pro-inflammatory prostaglandins, as well as that of cytoprotective prostaglandins (prostacyclin), particularly in the stomach and kidneys, and of platelet thromboxane A₂. This dual action explains, on the one hand, the anti-inflammatory effects, and on the other hand, certain adverse gastrointestinal and renal effects (Scheen, 2022).

I.3.2. Steroidal anti-inflammatory

For both chronic autoimmune diseases and acute inflammatory flare-ups, corticosteroids like prednisone and dexamethasone continue to be essential treatments. By binding to glucocorticoid receptors, they decrease the production of pro-inflammatory genes like NF- κ B and AP-1 and induce anti-inflammatory mediators, thereby modulating numerous pathways. By reducing the generation of ROS by activated immune cells, this widespread immunosuppressive impact indirectly reduces oxidative stress (**Figure 1**).

They have been shown to have strong anti-inflammatory effects in systemic autoimmune disorders, alcoholic hepatitis, asthma, and inflammatory bowel disease. Long-term corticosteroid use is limited by well-established side effects, such as immunosuppression, osteoporosis, hyperglycemia, and adrenal suppression, despite their significant efficacy (Manful *et al.*, 2025).

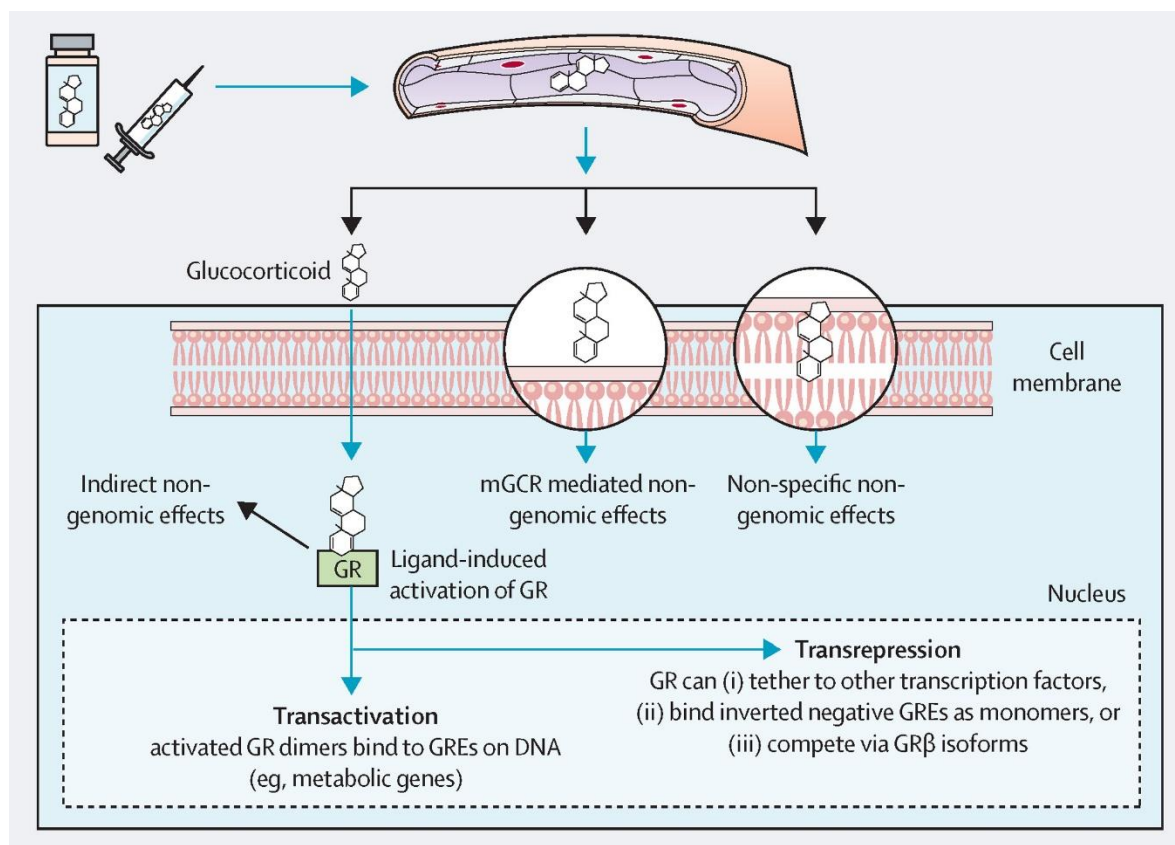


Figure 1. Mechanism of action of glucocorticoids (Buttgereit & Tam, 2026).

II. Oxidative stress

The idea of biological oxidative stress was expanded upon in "Oxidative stress: a concept in redox biology and medicine" and it was described as "a disruption of redox signaling and control and/or molecular damage, resulting from an imbalance between oxidants and antioxidants in favor of the oxidants"(Chen *et al.*, 2024). Oxidative stress results from an imbalance between the body's capacity to detoxify free radicals or repair the harm they cause. Reduced antioxidant defenses, increased formation of free radicals, or both may be the cause of this imbalance. Because free radicals react with essential biological components including lipids, proteins, and DNA, oxidative stress is harmful (Chandimali *et al.*, 2025).

II.1. Free radicals

A molecule that may exist on its own and has an unpaired electron in one of its atomic orbitals is known as a free radical (**Table 2**). What sets a free radical apart from other compounds is this unpaired electron. Because of this unpaired electron, free radicals are highly reactive and can easily interact with other molecules. A molecule may become a free radical by losing one of its electrons when it comes into contact with a free radical. This initiates a series of events that cause extensive cell damage. This harm can interfere with regular cellular processes and lead to a number of illnesses. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), are two of the most prevalent and important forms of free radicals in biology. ROS and RNS can be categorized into two groups: radicals and non-radicals. Radicals are molecular species containing at least one unpaired electron in their outer electron shells, rendering them highly reactive and capable of independent existence. Non-radical species, while not free radicals themselves, have the potential to undergo redox reactions and contribute to oxidative stress in living systems (Pooja *et al.*, 2025).

Table 2. List of free radicals and their reactivity (Arulselvan *et al.*, 2016)

No.	Free radicals	Reactivity
1	Superoxide anion	Generated in mitochondria, cardiovascular system, and other cell types
2	Hydrogen peroxide	Formed in the human body by a large number of reactions and yields potent reactive species
3	Hydroxyl radical	Highly reactive and generated during iron overload and such conditions in the human body
4	Peroxyl radical	Reactive and formed from lipids, proteins, DNA, and sugar molecules during oxidative damage
5	Nitric oxide	Neurotransmitter and blood pressure regulation and can yield potent oxidants during pathological states
6	Peroxynitrite	Highly reactive and formed from NO and superoxide
7	Ozone	Present as an atmospheric pollutant and can react with various molecules

II.2. Sources of free radicals

Free radicals and other reactive oxygen species are produced by the body's regular, vital metabolic activities or by external factors such exposure to ozone, x-rays, cigarette smoke, air pollution, and industrial toxins (Sultan, 2014).

II.2.1. Internal sources

These could be enzymatic processes that produce free radicals. These include the processes related to phagocytosis, the respiratory chain, and both in the cytochrome P 450 system and prostaglandin production.

Mitochondria, xanthine oxidase, phagocytes, iron and other transition metal processes, peroxisomes, arachidonate pathways, ischemia/reperfusion, and inflammation are a few internal sources of free radical production (Sultan, 2014).

II.2.2.External sources

These include oxygen's non-enzymatic interactions with organic substances. Ionizing radiation-induced processes also produce free radicals. Cigarette smoke, environmental pollutants, radiation, UV light, ozone, certain medications, pesticides, anesthetics, and industrial solvents are some examples of external sources of free radicals (Sultan, 2014).

II.2.3.Physiological factors

The development of free radicals is also caused by medical disorders and mental states such as stress, emotion, etc (Sultan, 2014).

II.3.Reactive oxygen species

ROS can be either free radicals with unpaired electrons or non-radical species created by radical–radical interactions (**Table 3**). They are mostly created by the incomplete reduction of molecular oxygen during normal metabolism. ROS can harm macromolecules because of their high reactivity, including proteins, lipids, and DNA. ROS are constantly produced by a variety of enzymatic processes and play crucial roles in redox signaling as second messengers. The activities of transcription factors (including AP-1, NF- κ B, and HIF-1) and other enzymes (such protein tyrosine phosphatases) are influenced by their interactions with redox-sensitive amino acid residues, like cysteine. Proliferation, differentiation, growth arrest or senescence, and cell death are among these impacted cellular processes (Anwar *et al.*, 2026).

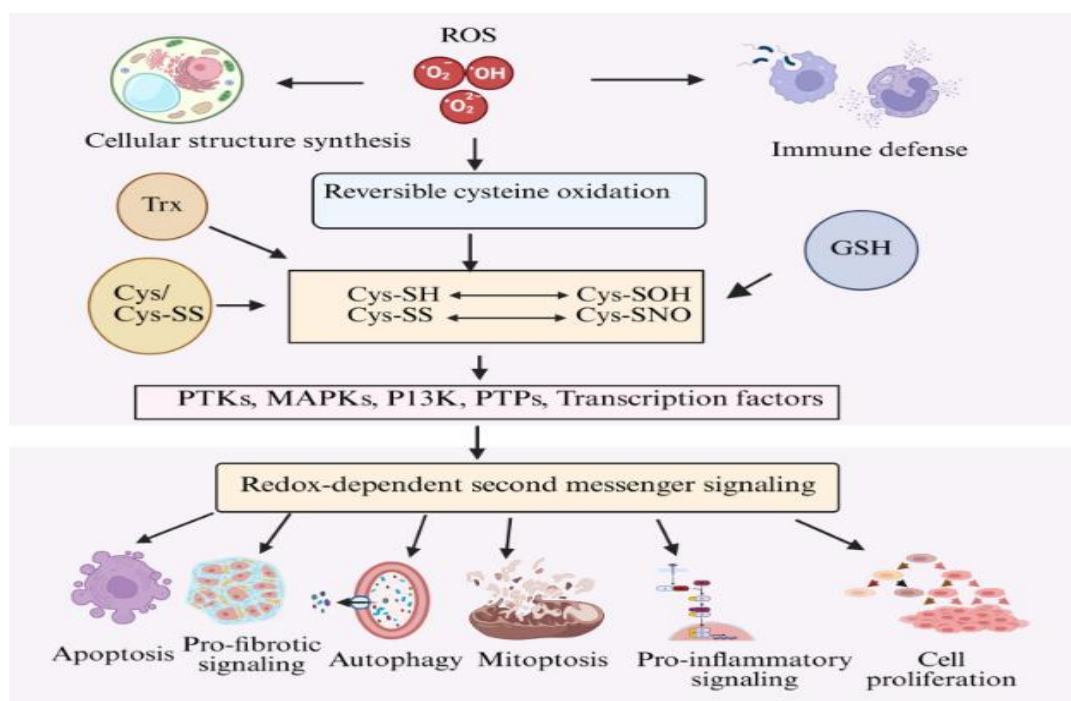
By causing the reversible oxidation of cysteine residues, physiological ROS function as second messengers. Kinases, phosphatases, and transcription factors are all affected by these redox switches, which are controlled by the thioredoxin (Trx), glutathione (GSH), and Cys/CySS systems. Thus, they regulate important functions such cell proliferation, pro-inflammatory/pro-fibrotic signaling, apoptosis, autophagy, mitosis, immunological defense, and structure synthesis (Anwar *et al.*, 2026).

II.4.ROS induced oxidative damage

Numerous cellular processes may be hampered by the constant intake and production of ROS from both endogenous and exogenous sources, which causes oxidative damage to cellular components (**Figure 2**). Proteins, enzymes, lipidic membranes, and DNA are the biological targets most susceptible to oxidative damage (Engwa, 2018).

Table 3. Important reactive species in biological system (Biswas, 2016).

Free radicals	Nonradicals
<i>Reactive oxygen species</i>	
Superoxide, $O_2^{\bullet -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\bullet}$	Singlet oxygen, $O_2^1\Delta g$
Peroxyl, $RO_2^{\bullet}$	Organic peroxides, $ROOH$
Alkoxy, $RO^{\bullet}$	Peroxynitrite, $ONOO^-$
Carbonate, $CO_3^{\bullet -}$	Peroxynitrous acid, $ONOOH$
<i>Reactive chlorine species</i>	
Atomic chlorine, $Cl^{\bullet}$	Hypochlorous acid, $HOCl$
	Chlorine gas, Cl_2
	Nitryl (nitronium) chloride, NO_2Cl
<i>Reactive nitrogen species</i>	
Nitric oxide, $NO^{\bullet}$	Nitrous acid, HNO_2
Nitrogen dioxide, $NO_2^{\bullet}$	Nitrosyl cation, NO^+
	Nitroxyl anion, NO^-
	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynitrous acid, $ONOOH$
	Alkyl peroxynitrites, $ROONO$

**Figure 2.** Physiological ROS act as second messengers by inducing reversible cysteine oxidation (Anwar et al., 2026).

II.4.1. Lipid peroxidation

The organisms most susceptible to oxidation are membrane phospholipids and polyunsaturated fatty acids. Lipids change into hydroperoxides, which can further oxidize and divide into alkanes and aldehydes. The peroxy radical, on the other hand, can produce a variety of hazardous aldehydes, including malondialdehyde (MDA), which is easily spread and has a longer half-life than RLs. It can bind to the basic components of DNA. Thus, a single oxidation can alter several lipid molecules and lead to a build-up of hydroperoxides in membranes, which would reduce their fluidity and transmembrane protein activity (Cotticelli *et al.*, 2013).

II.4.2. Nucleic acid

Through oxidation, RNS and ROS can harm nucleic acids. Due to its proximity to the primary source of free radicals, mitochondrial DNA is generally more susceptible to oxidative damage than nuclear DNA. All DNA components are directly impacted by ROS, especially the hydroxyl radical ($\text{OH}^\bullet$), which can result in single and double strand breaks, among other changes. In addition to creating cross-links between proteins and DNA, this radical can extract hydrogen to produce a number of modified purine and pyrimidine byproducts (Tvrdá & Benko, 2020). ROS causes site-specific DNA damage by oxidizing 2-deoxy-guanosine to 8-hydroxy-2deoxyguanosine (8-oxo-dG). 8-oxo-dG is a marker for DNA damage and oxidative stress (Reddy, 2023).

II.4.3. Proteins

Proteins are key targets for ROS attack, particularly by the $\text{OH}^\bullet$, $\text{RO}^\bullet$, and nitrogen-reactive radicals, which cause damage. Except for proteins with SH groups, hydrogen peroxide and superoxide radicals have minimal effects on proteins. After interacting with ROS, proteins may experience indirect damages like peroxidation, degradation, and fragmentation as well as direct damages like destroying particular amino acid residues and altering their tertiary structures. Loss of enzyme activity and changed cellular functions are the effects of protein damage. Keto, aldehydes, and carbonyl chemicals are typically the byproducts of protein oxidation (Engwa, 2018).

II.5. Reactive nitrogen species

Nitric oxide ($\text{NO}^\bullet$), peroxynitrite (ONOO^-), and nitrogen dioxide ($\text{NO}_2^\bullet$) are examples of reactive nitrogen species (**Figure 3**). These species are produced when nitric oxide ($\text{NO}^\bullet$) reacts with other molecules, especially radicals originating from oxygen. Neurotransmission, immunological control, and vasodilation are only a few of the physiological processes in which RNS are engaged.

Similar to ROS, dysregulated RNS levels can cause oxidative stress and play a role in the development of a number of illnesses, such as inflammation, neurodegeneration, and cardiovascular conditions (Pooja *et al.*, 2025).

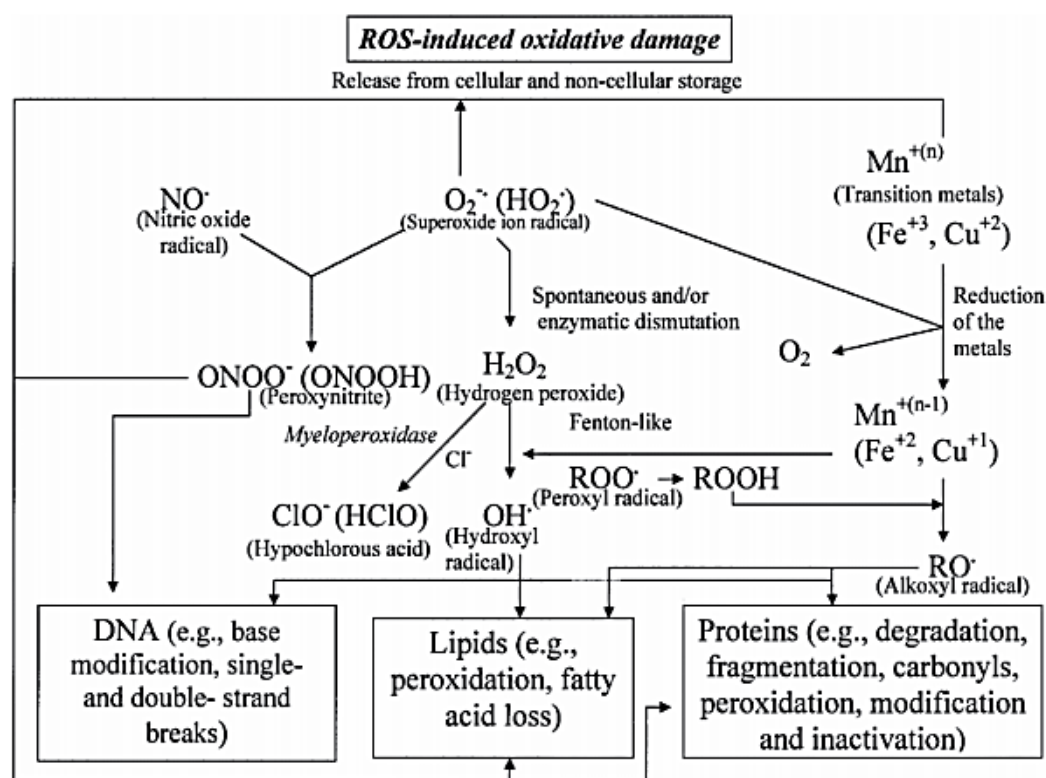


Figure 3. Oxidative damage inducing by ROS (Engwa, 2018).

II.6. Oxidative stress and human diseases

Oxidative stress contributes to the development and progression of various diseases, such as diabetes, obesity, and Alzheimer's. The structural alteration of proteins is significantly influenced by ROS and RNS. lipids and nucleic acids. The pathological development of diabetes, cancer, and cardiovascular illnesses is influenced by the latter structural alterations of proteins and nucleic acids. Additionally, increased AGE production is a result of oxidative stress. Consequently, RAGE is overexpressed. Binding AGEs to RAGE can worsen oxidative stress by triggering signaling cascades that release inflammatory cytokines and nuclear transcription factors. This interaction is linked to the development of pancreatic cancer in diabetes and obesity (Reddy, 2023).

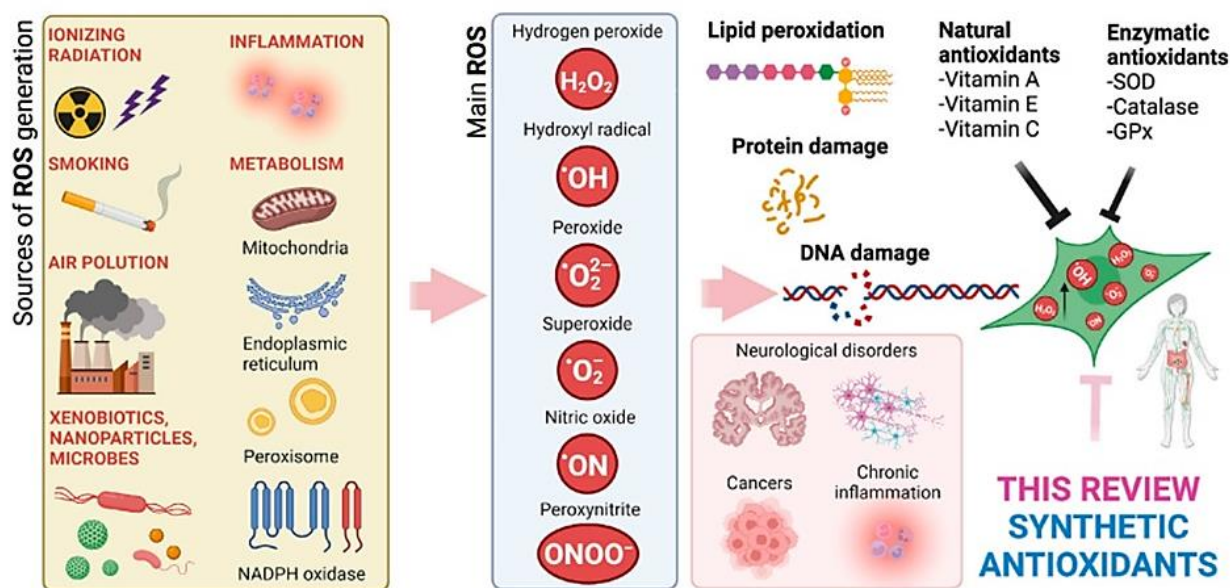


Figure 4. Sources of ROS/RNS (Tauchen *et al.*, 2025).

II.7. System antioxidant

A chemical that can prevent oxygen-mediated oxidation of a variety of materials, including simple molecules, polymers, and complex biological systems. Antioxidants are substances that considerably slow down or prevent the oxidation of a substance when they are present in lower concentrations than those of oxidisable substrates. Plants contain antioxidants that act as radical scavengers, converting them to less reactive forms species that react (Sultan, 2014).

Organisms have developed a sophisticated, three-line antioxidant defense system to combat the detrimental effects of ROS (Jomova *et al.*, 2024) .

- Antioxidant enzymes are part of the first-line defensive mechanism.
- Exogenous diet-derived small-molecule antioxidants are part of the second-line antioxidant defense pathway.
- Numerous enzyme systems fix or eliminate oxidized proteins and other macromolecules, ensuring the third-line antioxidant defense .

II.7.1. Enzymatic antioxidant systems

Is the most effective and uses antioxidant enzymes like glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (**Figure 5**) (Jomova *et al.*, 2024).

- **SOD**

Superoxide dismutases (SODs) protect cells by turning the superoxide radical (O_2^-) into oxygen and hydrogen peroxide (H_2O_2) through the alternating oxidation-reduction of metal ions in their active site (Younus, 2018).

- **Catalase**

Catalases and peroxidases are oxidoreductases found in almost all aerobic organisms, crucial for limiting oxidative stress from the natural production of H_2O_2 . Catalase, usually a 200–340 kDa homotetramer, efficiently catalyzes the heterolytic cleavage of the O–O bond in H_2O_2 into water and oxygen, thus maintaining the cellular redox balance and preventing the formation of ROS. (Jomova et al., 2024).



- **Glutathione peroxidase (GPx)**

The glutathione peroxidase (GPx) family comprises eight isoforms (GPx1–GPx8) with diverse biological roles; GPx1, the most abundant, is a tetrameric enzyme found in the cytoplasm of all organs, especially the heart, and each subunit contains a redox-sensitive selenocysteine. Together with SOD and catalase, GPx forms the first line of defense against oxidative stress by reducing hydroperoxides (like H_2O_2) to water, while oxidizing glutathione (GSH) to its disulfide form (GSSG), thereby maintaining cellular redox balance (Jomova et al., 2024).

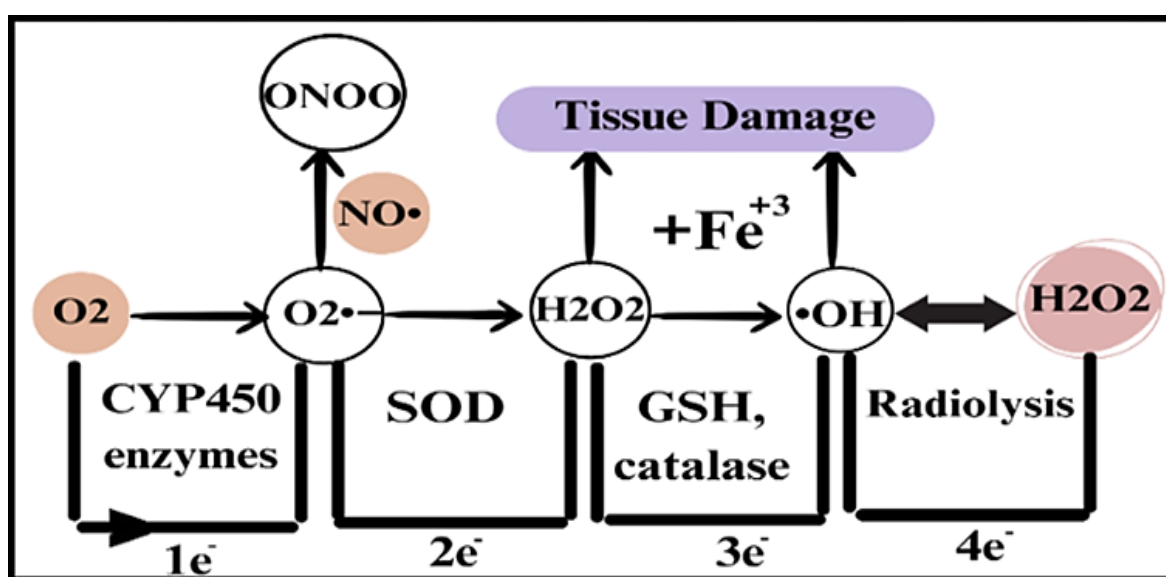
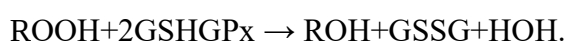


Figure 5. Process of generation of free radical (CYT P450 . SOD .GSH) (Pooja et al., 2025)

II.7.2. Non-enzymatic antioxidant systems

Reactive oxygen species (ROS), nitrogen (RNS), and sulfur (RSS) can be neutralized by non-enzymatic antioxidants, a wide family of bioactive substances that do not require enzymatic catalytic activity. Additionally, let antioxidant enzymes do a better job of eliminating ROS and shielding cells from oxidative harm. By shielding cellular macromolecules including lipids, proteins, nucleic acids, and carbohydrates from the irreversible oxidation linked to cellular aging and the pathogenesis of numerous chronic and degenerative diseases, these chemicals play a crucial role in preserving redox homeostasis. Endogenous and exogenous non-enzymatic antioxidants can be distinguished based on where they come from (**Table 4**). The body produces endogenous antioxidants, such as glutathione (GSH), uric acid, bilirubin, melatonin, and coenzyme Q10, as part of its defense mechanisms against oxidative stress. Vitamins C and E, carotenoids (β -carotene, lycopene), polyphenols (resveratrol, quercetin, catechins), flavonoids, trace minerals (selenium, zinc, copper), and phenolic compounds of plant origin are examples of exogenous substances that are mostly received by diet or supplementation. These substances can be categorized functionally in addition to their origin, according to their redox characteristics, solubility (fat-soluble or water-soluble), and capacity to interact with various cellular compartments. These antioxidants typically work by giving unstable oxidizing entities electrons or hydrogen to change them into less reactive forms and stop oxidation reaction chains from forming (M. de Oliveira *et al.*, 2026).

Table 4. Non-enzymatic antioxidants (Mirończuk-Chodakowska et al., 2018).

Intra- and extracellular non-enzymatic antioxidants.

Non-enzymatic antioxidants	
Intracellular	Extracellular
Ferritin	Transferrin
Myoglobin	Lactoferrin
Metallothioneins	Albumin
Coenzyme Q10	Ceruloplasmin
Glutathione	Uric acid
Melatonin	Bilirubin
Polyamines	

II.7.3. Pro-oxidant effect of antioxidant under certain conditions

Under some circumstances, antioxidants may also function as pro-oxidants. For instance, when ferric iron concentrations are high, ascorbate has a powerful enhancer of lipid peroxidation. According to recent research, ascorbate may occasionally exacerbate human DNA damage. Vitamin E function extends beyond its traditional antioxidant, neutral, or prooxidant action, according to recent mechanistic research on the early stages of LDL oxidation. Additionally, beta-carotene may act as a prooxidant in smokers lungs (Sultan, 2014).

III. Inflammation and oxidative stress: relationship and dependence

A fundamental, self-amplifying mechanism in the pathophysiology of many chronic diseases is the interaction between inflammation and oxidative stress. When they are present in excess, ROS behave as both vital signaling molecules and mediators of cellular harm. Redox equilibrium is upset, biomolecular damage is encouraged, and pro-inflammatory signaling cascades are triggered by dysregulated ROS levels. On the other hand, inflammation creates a vicious cycle of persistent tissue damage and disease progression by increasing ROS formation through immune cell activation and redox-sensitive pathways. The main molecular pathways connecting oxidative stress and inflammation are described in depth in the following sections (Manful *et al.*, 2025).

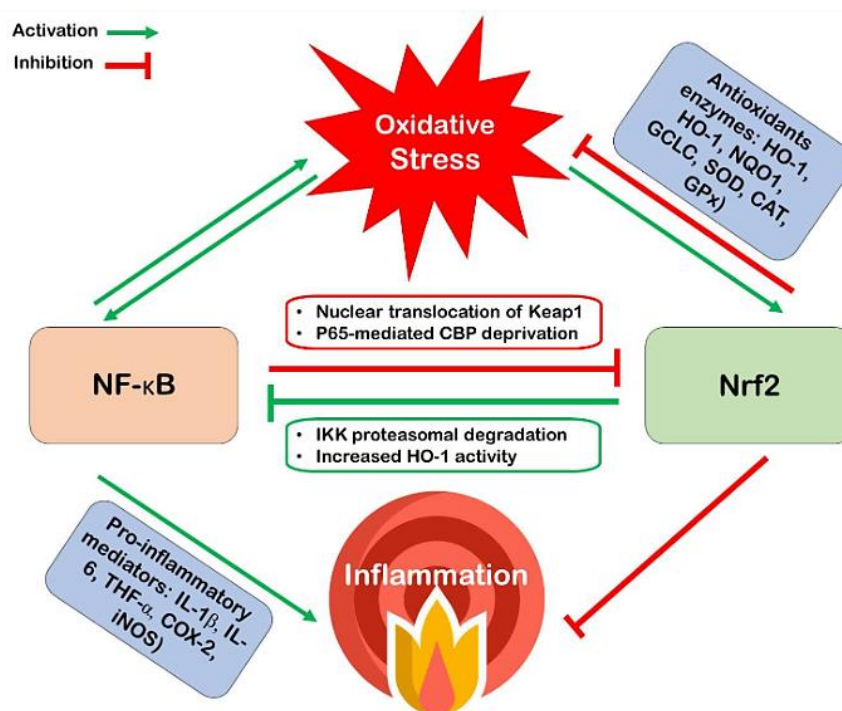


Figure 6. Cross-talk between NF- κ B and Nrf2 signaling pathways (Manful *et al.*, 2025).

Chapter II:

***Cistanche tubulosa* plant**

***Cistanche tubulosa* plant**

I. *Cistanche tubulosa* “Ginseng of the Desert”

One of the most widely utilized herbs in the *Cistanche* family, *Cistanche tubulosa*, is referred to as the "Ginseng of the Desert" due to its numerous health benefits (Liu *et al.*, 2023).

I.1. Taxonomy

The taxonomic classification of *Cistanche* is summarized in **Table 5**.

Table 5. *Cistanche* Taxonomy (Aldughayman *et al.*, 2024).

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Orobanchaceae
Genus	<i>Cistanche</i>

I.2. Botanical description

Members of the Orobanchaceae family are often succulent and covered in scale leaves. Both the leaves and the stem are chlorophyll-free and parasites on the roots of a variety of plants. According to reports, the plants are either holo- or hemi-parasitic, annual or perennial herbs or shrubs that grow on the roots of their hosts. The plants are succulent and covered with small, membranous, simple, sessile leaves grouped in a spiral pattern. There are no well-developed stomata, only a hydathode for gas and water vapour exchange. Flowers are arranged in spikes. The flowers are bracteate and tiny, appearing in the axils of a scale . The parasite's peduncle begins crimson and gradually turns yellow or white yellow as it matures (KARAK, 2010).

Cistanche tubulosa, a perennial parasitic plant belonging to the Orobanchaceae family, grows in arid regions of Asia and Africa. It has been found in Saudi Arabia (Sakaka desert, Aljouf, KSA), China, India, and Japan .

The stem is succulent and fleshy with a high water content, and it goes by several common names in Chinese medicine, including Desert Hyacinth, Desert Ginseng, and Rou Cong Rong (Musa *et al.*, 2021). *C. tubulosa* is a yellow-brown herb with a single, unbranched, upright, glabrous stem that can grow up to 60 cm tall (**Figure 7**). It has been documented from China, Pakistan, and other locations (KARAK, 2010).



Figure 7. *Cistanche tubulosa* photography

I.3. Phytochemical content and biological activities

Cistanche contains both volatile and non-volatile compounds, including as lignans, phenylethanoid glycosides, oligo- and polysaccharides, alkaloids, and iridoids. *Cistanche*'s diverse phytochemical content and biological activity make it highly valued in Chinese folk and traditional medicine. It has been used as an aphrodisiac for impotence and infertility, as a laxative for senile constipation, and for neuroprotection against Alzheimer's, Parkinson's, and depression, as well as anti-aging, anti-neoplastic, antiplatelet aggregation, antifungal and antibacterial, hepatoprotective, immunostimulant, antioxidant, renal support, and antitumor in colorectal esophageal carcinoma. It is used to treat psychroalgia of the knees and back, boost immunity, improve cognitive function, and act as an antidepressant and had hypocholesterolemic effect. According to a literature survey *Cistanche* is a non-toxic herb that can be used safely in the long run. *C. tubulosa* is known for its therapeutic properties, particularly in Chinese traditional medicine.

However, the volatile ingredients have not been extensively researched and their chemical compositions are unknown (Musa *et al.*, 2021).

Chapter III:

Materials and methods

MATERIALS AND METHODS

I. Materials

I.1. Human blood

A healthy, nonsmoking volunteer donor who has not had any medical treatment for at least fifteen days is the source of fresh human blood for the study of anti-inflammatory activity.

I.2. Rat blood

Blood from healthy, untreated rats is collected for the study of antioxidant activity.

I.3. Plant material

The *Cistanche tubulosa* plant was gathered in April 2026 from the SIDI AMEUR region in M'sila. The flowering section was dried in the shade at ambient temperature, ground, and stored away from light before use.

I.4. Products and reagents

As chemical reagents for this study, we employed: 1,1-diphenyl-2-picryl-hydrazyl (DPPH), gallic acid, quercetin, folin reagent, physiological saline, trichloroacetic acid (TCA), Na₂CO₃, chlorure d'aluminium (AlCl₃), 2,2'-Azobis (2-methylpropionamidine) dihydrochloride (AAPH), ascorbic acid (L-ascorbic acid), diclofenac sodium, 2,6-Di-tert-butyl-4-methylphenol (BHT) are sourced from Fluka (France). The salts used to prepare the buffers are obtained from Panreac (Spain). The solvents are analytical grade and obtained from Riedel-de Haén, Prolabo, and Sigma.

II. Methods

II.1. Preparation of the aqueous extract

Cistanche tubulosa flowers aqueous extract is made using the technique of (Kasmi et al., 2017) a standardized decoction procedure was used to prepare the aqueous extract (Aq.E). In summary, 50 g of plant powder was soaked in 500 ml of distilled water and heated at 90 °C for 15 minutes while being continuously stirred. Whatman No. 1 filter paper was used to filter the mixture, and a drying oven was used to concentrate the filtrate at 40 °C. Until it was needed again, the finished extract was kept at 4 °C.

$$\text{Yield \%} = \frac{\text{Mass in grams of the resulting dry extract}}{\text{Mass in grams of dry plant material}} \times 100$$

II.2. Total polyphenol content

Folin-Ciocalteu reagent (FCR) is composed of two sodium salts (molybdate and tungstate), two inorganic acids (hydrochloric and phosphoric), lithium sulfate, and water. When reduced by phenolate ions, the phosphomolybdic/phosphotungstic acid combination takes on a vivid blue color. In its oxidant form, it appears yellow. Actually, the process of color creation relies on the transfer of electrons to diminish the acid complex and create chromogens with lower valence metals (Raposo *et al.*, 2024).

Briefly a modified FCR method was used to measure the total phenolic content (TPC) in *C. tubulosa* extracts (Elbouzidi *et al.*, 2023). After mixing extract aliquots (25 μ l) with 125 μ l of 10% FCR for four minutes, 100 μ l of 7.5% Na_2CO_3 was added. a UV-Visible Spectrophotometer was used to detect absorbance at 765 nm against a blank following a 90-minute incubation period at room temperature.

The concentration of total polyphenols is calculated from the regression equation of the calibration range (**Figure 8**) established with gallic acid (0 - 140 μ g/ml) and is expressed in μ g of gallic acid equivalent per milligram of extract (μ g GAE/mg of extract).

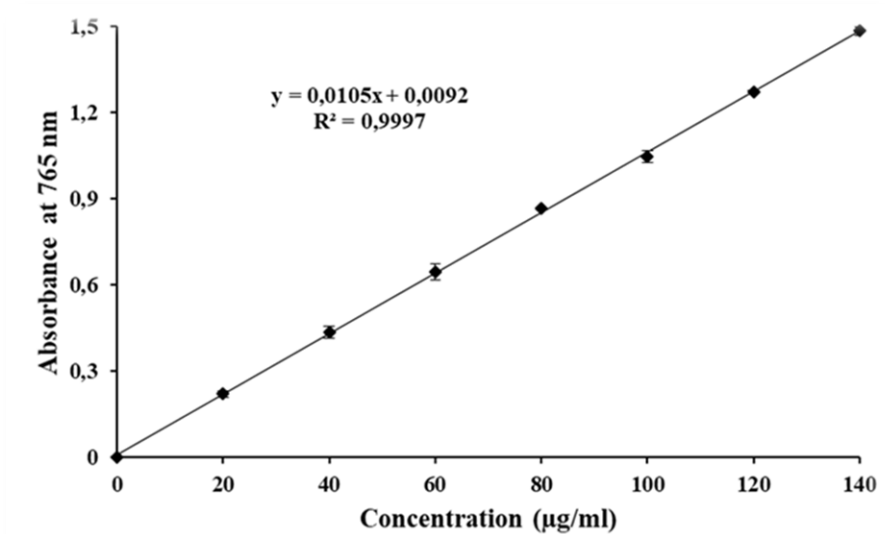


Figure 8. Calibration curve of gallic acid.

II.3. Total flavonoid content

A colorimetric method for measuring the amount of flavonoid derivatives in medications using aluminum chloride (AlCl_3). The development of an acid-stable combination combining the Al^{3+} cation, the flavonoid's C-4 keto group, and either its C-3 or C-5 hydroxyl group serves as the chemical basis for this technique. Furthermore, the vicinal hydroxyl groups in the B ring of flavonoids form acid-labile complexes with the AlCl_3 reagent (Sultana *et al.*, 2024).

An aluminum chloride colorimetric assay modified from (Madjitouloum Betoloum *et al.*, 2018) was used to measure the total flavonoid content (TFC) in *C. tubulosa* extracts. 150 µl of each extract aliquot was combined with 150 µl of a 2% AlCl₃ solution. Absorbance was measured at 430 nm against a blank following a 10 minute incubation period at room temperature. The concentration of flavonoids in the extracts is deduced from a calibration range established with quercetin (0-16 µg/ml) and expressed in micrograms of quercetin equivalent per milligram of extract (µg EQ/mg of extract) (**Figure 9**).

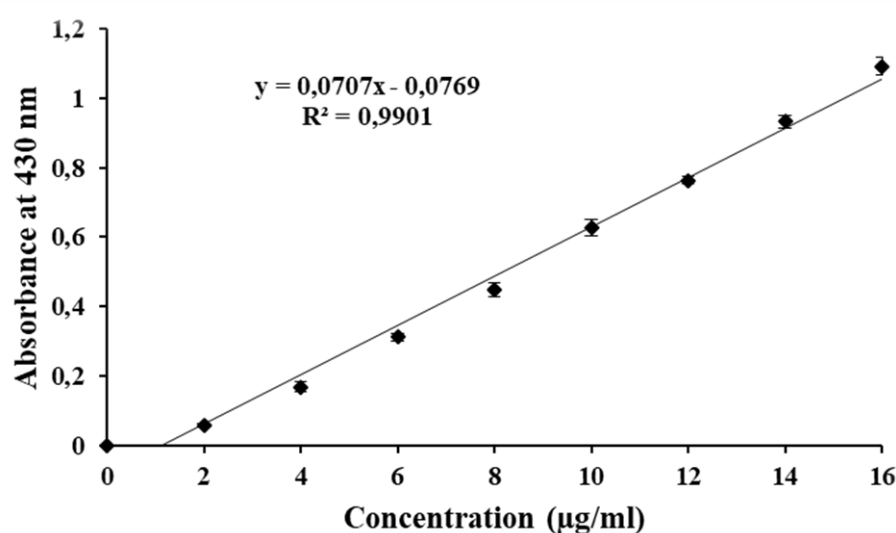


Figure 9. Calibration curve of quercetin.

II.4. Anti-inflammatory activity of extracts of *Cistanche tubulosa* flowers

II.4.1. Membrane stability of human erythrocytes

Human red blood cells are plentiful, pliable, and physically resemble lysosomal membranes. Maintaining lysosomal integrity stops the release of phospholipase A₂, which stops the breakdown of arachidonic acid and the production of prostaglandins, which are important mediators of inflammation. Therefore, substances that stabilize membranes can lessen inflammatory reactions. RBC membranes are a useful *in vitro* model for assessing anti-inflammatory efficacy because they resemble lysosomal membranes (Samaraweera *et al.*, 2023).

- **cell suspension of human red blood:**

An anti-cubital arterial puncture was used to draw blood (5 mL) from healthy volunteers in order to create the erythrocyte suspension. The precipitate of the centrifuged blood (3000 g; 10 min) was washed three times with NaCl solution (154 mM) in sodium phosphate buffer (10 mM; pH 7.4) to extract the red blood cells. Then resuspended in isotonic PBS solution to prepare a 10% (v/v) suspension of human red blood cells.

- **Determination of the membrane stability of human red blood cells**

The effect of *C.tubulosa* extracts on the stabilization of erythrocytes following the induction of hemolysis by a hypotonic solution is the basis for the investigation of the plant's in vitro anti-inflammatory activity. This was done using the red blood cell membrane stabilization technique outlined by (Fujiati *et al.*, 2022). In summary, 1.25 ml of each extract at varying doses (1, 2.3 mg/ml) dissolved in a hypotonic buffer (10 mM, pH=7.4, and 50 mM NaCl) or 1.25 ml of diclofenac sodium, which serves as a reference, are mixed with 125 µl of the erythrocyte suspension diluted to 10%. The erythrocyte suspension in the hypotonic medium alone is the control, which is regarded as 100% hemolysis. Centrifugation is performed for ten minutes at 3000 rpm following a 10-minute incubation period at room temperature. At 450 nm, the recovered supernatant's absorbance is measured. The following formula is used to determine the percentage of hemolysis inhibition:

$$\%inhibition\ of\ hemolysis = 100 \times \left(\frac{Abs\ control - Abs\ test}{Abs\ control} \right)$$

II.5.Antioxidant activity of extracts of *Cistanche tubulosa* flowers

II.5.1.Scavenger effect of the free radical DPPH[•]

Using DPPH[•] as a stable free radical, the free radical scavenging activity of *Cistanche tubulosa* extract was assessed using the procedure outlined by (Zbadi *et al.*, 2018). Purple diphenyl picrylhydrazyl is reduced to yellow diphenyl picrylhydrazine in this experiment by the antioxidant acting as a proton donor; the antioxidant's strength is inversely related to its antioxidant activity. 150 µl of the extract or standard (BHT) solutions at varying concentrations are combined with 150 µl of the DPPH[•] (0.1 mM) solution. After 30 minutes of dark incubation, a microplate reader is used to measure the absorbance at 517 nm. All of the reagents are present in the control, with the exception of the test sample, which is swapped out for an equivalent volume of methanol.

The following formula is used to calculate the antiradical activity:

$$\%Anti - radical activity = 100 \times \left(\frac{Abs\ control - Abs\ test}{Abs\ control} \right)$$

II.5.2.Reducing power

The FRAP assay is based on the quick reduction of ferric-tripyridyltriazine (Fe^{3+} -TPTZ) in samples by antioxidants, producing the blue-colored product ferrous-tripyridyltriazine (Fe^{2+} -TPTZ). A standard curve was created by adding the FRAP reagent to a range of known Fe^{2+} solution concentrations. This allowed the Fe^{2+} concentration of the samples to be ascertained, hence demonstrating "antioxidant capacity. The method outlined by (Madhuranga, 2023) was used to assess the reducing power of *Cistanche tubulosa* extracts. First, 625 μ l of phosphate buffer (0.2 M, pH 6.6) and 625 μ l of 1% $K_3Fe(CN)_6$ are combined with solutions of extracts and reference antioxidant (BHT) at varying concentrations. 625 μ l of TCA (10%) and 125 μ l of $FeCl_3$ (0.1%) are added to the reaction medium after 20 minutes of incubation at 50°C and 15 minutes of chilling. The absorbance is measured at 700 nm in comparison to a blank without $FeCl_3$. The reducing power and absorbance are intimately correlated. The absorbance versus sample concentration curve is used to get the EC_{50} value.

II.5.3.AAPH induced erythrocyte oxidative hemolysis assay (Anti-hemolytic activity)

The water-soluble azo compound AAPH breaks down around 37 °C to produce carbon-centered radicals, which quickly transform into peroxy radicals when oxygen is present. These peroxy radicals start lipid peroxidation chain reactions, which cause endogenous antioxidants to be depleted and ROS to build up. AAPH offers a continuous and controlled free radical flux in contrast to H_2O_2 , which breaks down quickly and causes a brief oxidative burst. This makes it especially useful for simulating circumstances of chronic oxidative stress. AAPH has been utilized extensively in a variety of cell types (Zhang *et al.*, 2026).

To evaluate the extracts, 60 μ L of each concentration (5-40 μ g/mL) was combined with 120 μ L of a rat erythrocyte solution (1% in PBS, pH 7.4). After 30 minutes of incubation, the mixture was combined with 120 μ L of AAPH (120 mM). For the control and standard, the extracts were swapped out for saline (0.9% NaCl) or ascorbic acid, respectively. A microplate reader was used to test the mixture's absorbance at 450 nm every ten minutes. Half hemolysis times were used to measure the preventive effect of *C. tubulosa* extract against erythrocyte hemolysis caused by free radicals (Bouhlali *et al.*).

II.6. Statistical analyzes

The results of the assays are expressed as mean $\pm$ SD. The IC₅₀ , EC₅₀ and HT₅₀ values were calculated from the curve [% = f (concentrations)]. Multiple comparisons and the determination of significance levels were performed using T-test and one-way ANOVA followed by Tukey's test. Differences were considered statistically significant at the 0.05 level ($p \leq 0.05$).

Results and discussion

Results and discussion

I. Results

I.1. Preparation of *Cistanche tubulosa* extract

The resulting aqueous extract appears as a fine, brown, hygroscopic powder. The yield is expressed as a percentage of the mass of the dry extract relative to the mass of the dry plant powder.

- The aqueous extraction yield is 26%

I.2. Total polyphenol and flavonoid levels

The Folin-Ciocalteu and aluminum trichloride techniques were used to determine the total polyphenol and flavonoid content of *Cistanche tubulosa* flowers extract, respectively. The findings demonstrate the aqueous extract's high levels of flavonoids and total polyphenols (**Table 6**) .

Table 6. Total polyphenol and flavonoid content of aqueous extract of *Cistanche tubulosa* flowers

Extract	Polyphenols	Flavonoids
	µg gallic acid equivalent / mg extract	µg quercetin equivalent / mg extract
Aq.E	56,74 ± 1,54	10,54 ± 0,47

The values represent the average of 3 trials ± SD.

I.3. Anti-inflammatory activity of *Cistanche tubulosa* flowers extract

The *C.tubulosa* flowers extract's *in vitro* anti-inflammatory action, which is based on research on the extract's ability to prevent red blood cell hemolysis brought on by a hypotonic environment, is shown in the (**Figure 10**).

The results obtained show that the extract significantly protects ($p < 0.001$) human erythrocytes against hemolysis. At a concentration of 3 mg/ml, the aqueous extract exhibited the highest rate of hemolysis inhibition (100%) compared to 97.88% with diclofenac sodium.

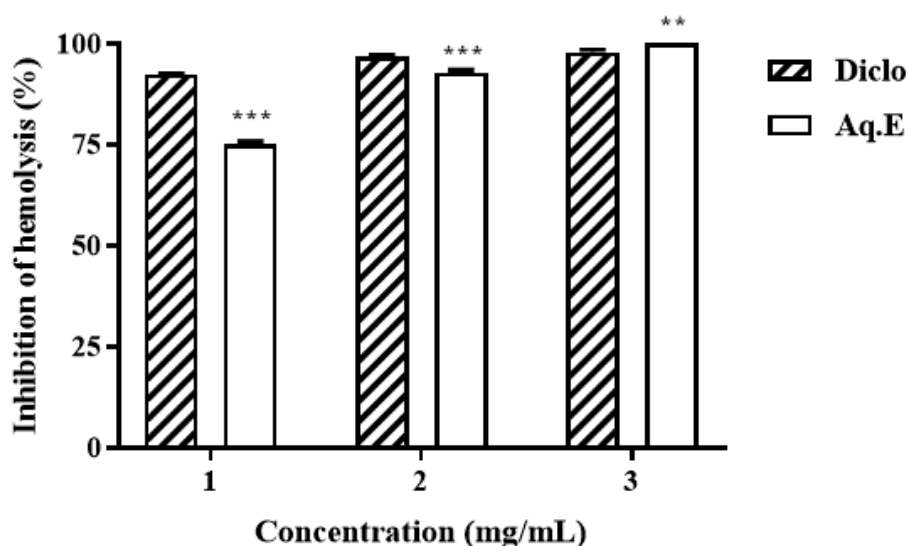


Figure 10. Effect of aqueous extract (Aq.E) of *Cistanche tubulosa* flowers on hypotonic-induced lysis of human erythrocytes. Values represent mean $\pm$ SD (n=3). ***: $p < 0.001$; **: $p < 0.01$ vs diclofenac.

I.4. Antioxidant activity of *Cistanche tubulosa* flowers extract

I.4.1. DPPH radical scavenging effect

The findings demonstrate the concentration-dependent antiradical activity of the aqueous extract of *Cistanche tubulosa*'s floral portion (**Figure 11**). A maximal activity of 82,29% is required reaching a concentration of 600 μ g/ml. Compared to the typical antioxidant BHT, which has a maximum activity of 94 % at a concentration of 200 μ g/ml, which is more effective. **Table 7** shows the 50% inhibitory concentrations (IC_{50}) of extract and BHT.

Table 7. IC_{50} values for *Cistanche tubulosa* flowers extract and BHT.

Extrait	IC_{50} (μ g/ml)
Aq.E	135,41 $\pm$ 0.95
BHT	30,88 $\pm$ 2,16

Values represent the mean of three trials $\pm$ SD

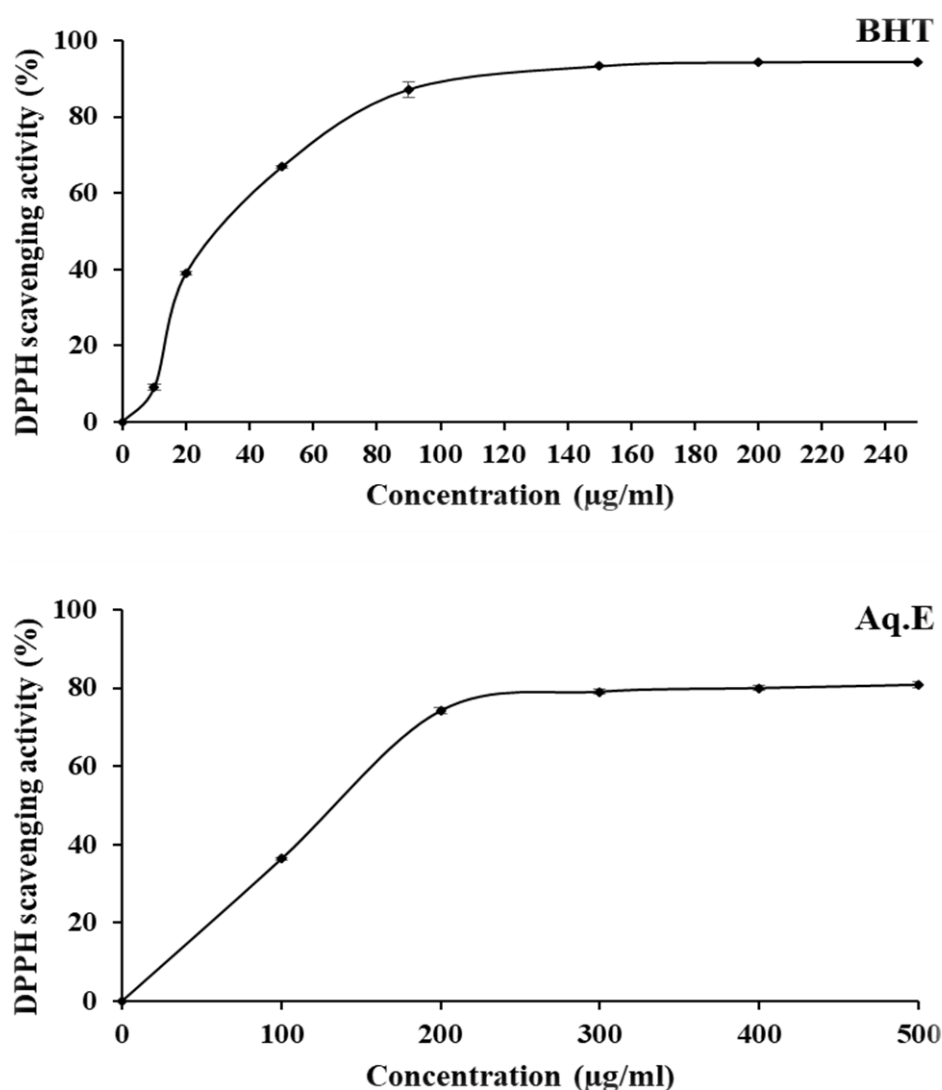


Figure 11. Antiradical activity of the Aq.E of *C.tubulosa* flowers against the DPPH[•] radical, compared to the reference antioxidant (BHT). Each value represents the mean of 3 assays $\pm$ SD.

I.4.2. Reducing power

The findings demonstrate the good reducing capability of the *Cistanche tubulosa* extract (**Figure 12**). In fact, the extract had the greatest reducing power at a concentration of 280 µg/ml, a concentration-dependent reducing power was also observed. At 60 µg/ml, BHT exhibits its highest reducing power. **Table 8** displays the EC₅₀ values.

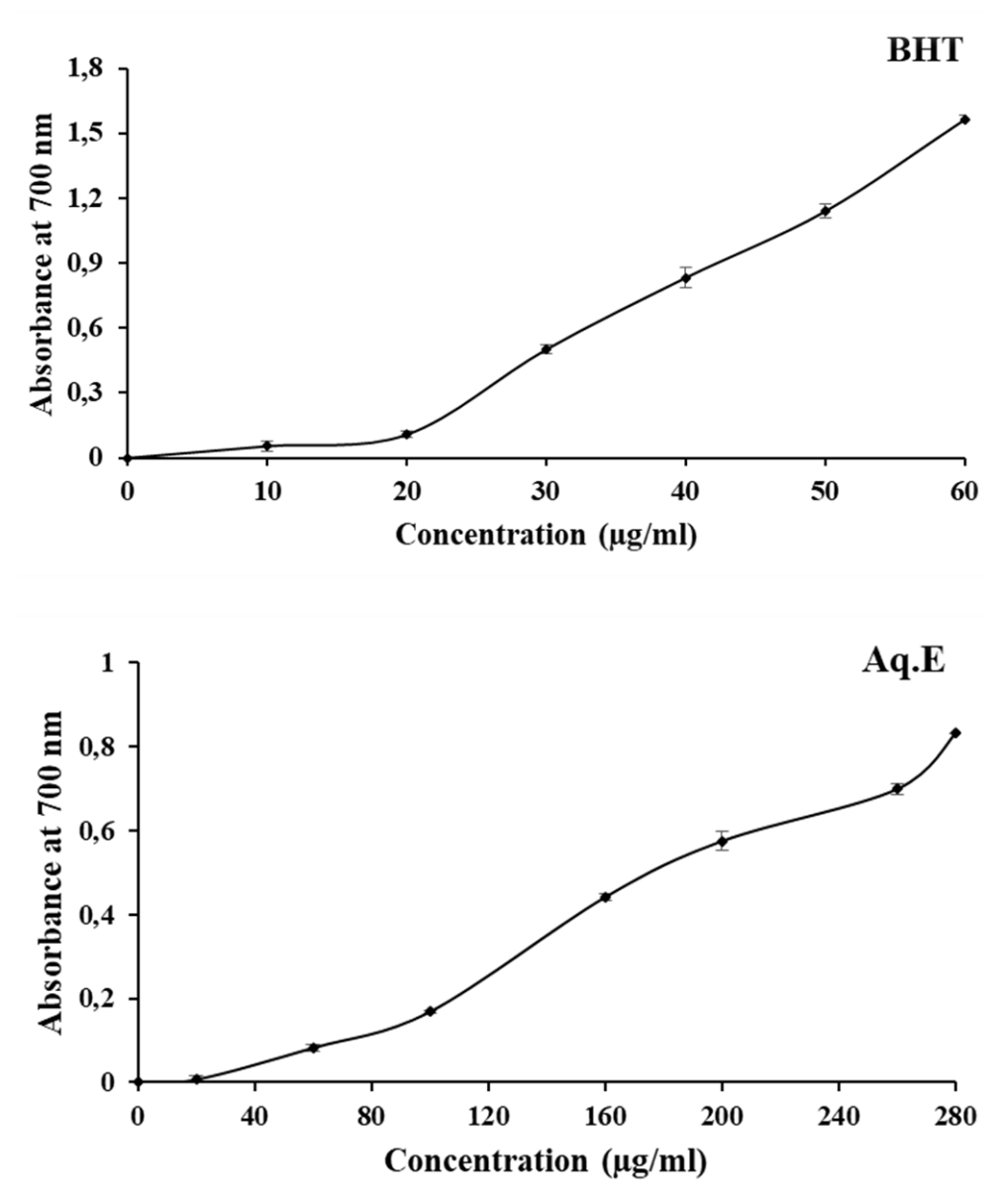


Figure 12. Reducing power of Aq.E of *C.tubulosa* flowers compared to the reference antioxidant (BHT). Each value represents the mean of 3 assays $\pm$ SD.

Table 8. EC₅₀ values for *cistanche tubulosa* extract and BHT.

Sample	EC ₅₀ (µg/ml)
Aq.E	178,86 $\pm$ 4,17
BHT	30,56 $\pm$ 0,79

Values represent the mean of 3 trials $\pm$ SD.

I.4.3. AAPH induced erythrocyte oxidative hemolysis assay

Erythrocytes pre-incubated with varying doses of *C. tubulosa* extract demonstrated a potent protective effect against erythrocyte lysis produced by AAPH. It also appears that there is a concentration-dependent effect of this extract on increasing the resistance of the erythrocyte cell membrane to free radical attack as it shown in **(Figure 13)**. At a concentration of 40 µg/ml, the aqueous extract leads to a decrease in erythrocyte hemolysis with half-hemolysis times (HT₅₀) of 68.80 ± 0.72 min. These results are less effective than those of ascorbic acid (HT₅₀ = 122.30 ± 0.22 min) used as a standard **(Table 9)**.

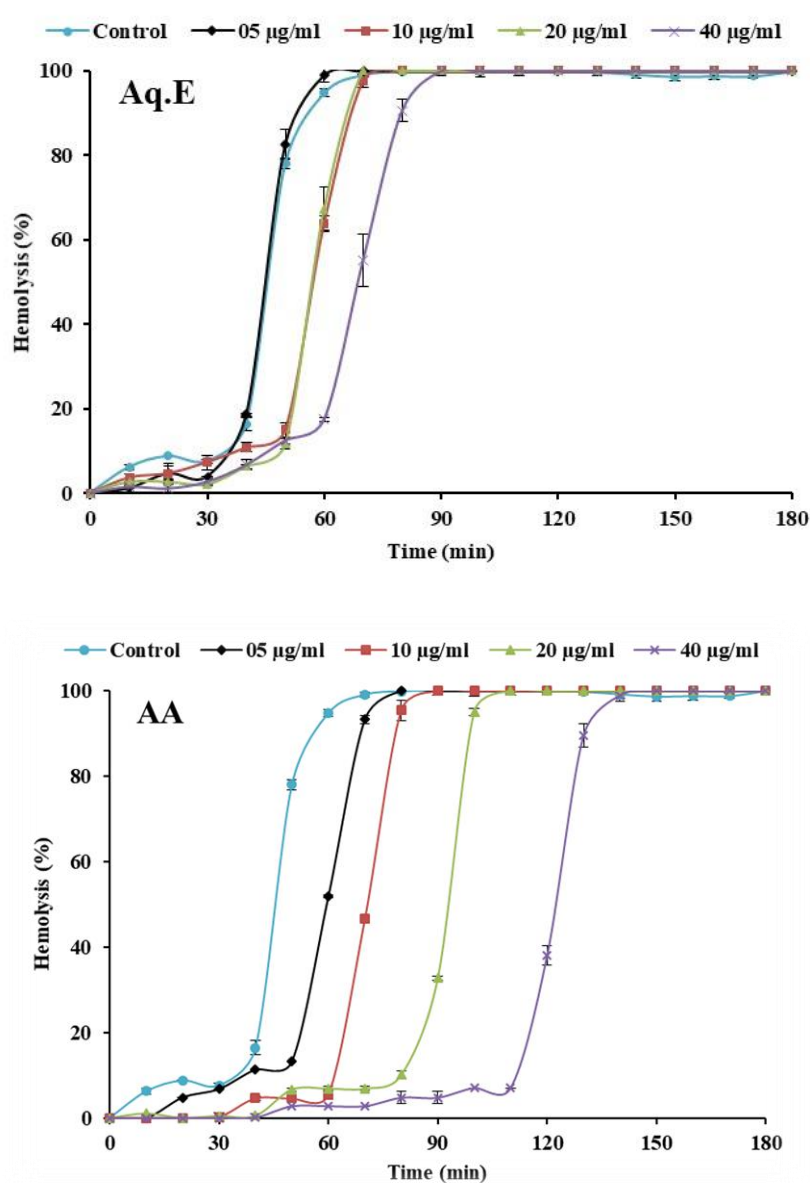


Figure 13. Anti-hemolytic activity of aqueous *C.tubulosa* flowers extract (Aq.E) and ascorbic acid. Each value represents the mean of 3 trials $\pm$ SD.

Table 9. The protective effect of *C. tubulosa* flowres aqueous extract (Aq.E) and Ascorbic acid (AA) against AAPH induced hemolysis of human erythrocytes.

Samples ($\mu\text{g/mL}$)	Hemolysis half-time (min)			
	05	10	20	40
Control	45.45 $\pm$ 0.23			
Aq.E	44.92 $\pm$ 0.30 ^{NS}	57.16 $\pm$ 0.22 ^{***}	57.82 $\pm$ 0.32 ^{***}	68.80 $\pm$ 0.72 ^{***}
AA	59.36 $\pm$ 0.75 ^{***}	70.67 $\pm$ 0.06 ^{***}	92.77 $\pm$ 0.10 ^{***}	122.30 $\pm$ 0.22 ^{***}

NS: not significant; ***P<0.001 compared with control. Values are mean $\pm$ SD (n = 3).

II. Discussion

Anti-inflammatory medications continue to be problematic since prolonged usage can result in gastrointestinal, cardiovascular, and other damage. Therefore, there is a chance to utilize safer natural nonsteroidal anti-inflammatory medications and discover an alternate medication for inflammation (Fujiati *et al.*, 2022). High dosages of antioxidant synthesis (BHA, BHT, and TBHQ) administered over an extended period of time may have hepatotoxic effects and damage renal tubules and glomeruli structurally, which may result in nephrotoxicity (Ren *et al.*, 2025). Due that researchers are looking for novel medical plant-based anti-inflammatory and antioxidant compounds that can replace synthetic ones.

The medicinal plant *Cistanche tubulosa*, which is mainly studied in its stems and isolated compounds, is known for its immunomodulatory, anti-inflammatory, and antioxidant qualities. Nevertheless, no peer-reviewed research has yet directly examined the anti-inflammatory properties of its blossoms. In order to close this gap, our research assesses *Cistanche tubulosa* flowers' anti-inflammatory properties as a novel contribution and their antioxidant properties as a supplementary feature.

II.1.Preparation and quantification of total polyphenols and flavonoids

Boiling plant material in a predetermined amount of water or a solvent is known as decoction. It is one of the most used methods for removing substances from medicinal plants, such as polyphenols, alkaloids, terpenoids, and flavonoids. One plant material or a combination of plant materials can be used to make a decoction (Charuka & Bandara, 2025). Despite that, traditional extraction methods, are uncomplicated yet slow, solvent-intensive, and prone to thermolabile chemical breakdown (Karthiraja *et al.*, 2025).

In the other hand, green extraction technology, as opposed to standard extraction technology, can avoid the presence of hazardous components in products and the environment by substituting green extraction solvents for toxic extraction solvents. Additionally, it can minimize the extraction time, lower solvent and energy consumption, and increase the extraction rate employing auxiliary extraction technologies (microwaves, ultrasonics, and pressure) (Cao *et al.*, 2025).

More over one of the most important elements in determining extraction efficiency is the choice of solvent. When choosing particular kinds of bioactive chemicals, the solvent's polarity is crucial: Water is a polar solvent that effectively extracts hydrophilic substances such anthocyanins, polyphenols, and flavonoids. The extraction of polyphenols is a good example of the importance of solvent polarity and the advantages of mixed solvent systems. Highly polar chemicals are mostly extracted by pure water, which emphasises the need to optimise solvent systems to match the polarity of the target bioactive molecules (Sun *et al.*, 2025).

Also drying and grinding are essential steps in the preparation process. Increasing the polysaccharides' surface area is the main goal of grinding, which makes it easier for solvents to enter the cells. Smaller particles provide a greater contact area, which encourages polysaccharide disintegration and release and improves extraction efficiency (Zhang *et al.*, 2025).

The yield and stability of bioactive chemicals are strongly impacted by both temperature and extraction time, Elevated temperatures have the potential to enhance mass transfer, solubility, and diffusion rates, hence improving extraction efficiency. However, thermolabile substances including vitamin C, polyphenols, and essential oils may undergo oxidative destruction after extended contact to high temperatures. In order to maximize the recovery of bioactive compounds, the extraction time is also highly important. Long extraction durations can boost output, but they can also cause oxidation, hydrolysis, or polymerization, which might change the bioactivity of delicate phytochemicals (Sun *et al.*, 2025).

Polyphenols are especially important because of their antibacterial, antioxidant, and disease-preventive qualities. Flavonoids and phenolic acids are two subcategories of these bioactive substances that are frequently found in plants and have a variety of medicinal uses (Tripathi & Suthar, 2025). Multiple hydroxyl (OH) groups are present in this family of substances, which range in structure from simple molecules to complex polymers (Silva *et al.*, 2026).

Flavonoids are secondary metabolic polyphenolic chemicals with a basic structure made up of three rings (C6–C3–C6), referred to as A, B, and C. Plants contain these substances in large quantities, both free (aglycones) and bound to sugars (glycosides). According to research, flavonoids have a variety of pharmacological actions, such as antioxidant and anti-inflammatory properties (Silva *et al.*, 2026).

Every component of *C.tubulosa*, including the tube root, stem, and flowers, contains phytochemicals such as carbohydrates, phenols, alkaloids, tannins, flavonoids, and phytosterols (Sharma & Pareek) .

The Total phenolic (TPC) and flavonoid (TFC) contents in aqueous roots extract from *C. phelypaea* are mentioned in recent research : TPC (92.45 ± 0.73 mg GAEs/g extract) and TFC (14.26 ± 0.54 mg QEs/g extract). Extracts made with hot water had higher TFC levels than extracts made at room temperature. This may be connected to flavonoids' high temperature solubility (Rahim *et al.*, 2022). Our findings after a decoction of dried powder of *cistanche tubulosa* flowers using distilled water as solvent indicate that the plant's floral portion contains a good concentration of polyphenolic chemicals : TPC ($56,74 \pm 1,54$ mg GAEs/g extract) and TFC ($10,54 \pm 0,47$ mg QEs/g extract) , which are responsible for the plant's anti-inflammatory and antioxidant properties. The variations in the Cistanche species utilised and the part used in addition of the geographic location where the plant was picked are responsible for the discrepancy between these findings.

II.2. Anti-inflammatory activity

II.2.1. Effect of extract on the membrane stability of human erythrocytes

Initially, the primary organelles that break down cells are called lysosomes. Lysosomes act as platforms for signaling pathways in addition to mediating the degradation of endogenous and endocytosed macromolecules, cellular components, and particles. Inflammasome activation is a detrimental effect of lysosomal membrane instability. By encouraging the maturation and secretion of pro-inflammatory cytokines like interleukin- 1β (IL- 1β) and IL-18, inflammasomes multiprotein complexes play a critical role in the innate immune response. Lysosomal dysfunction and subsequent inflammasome activation have been connected to other autoimmune diseases like multiple sclerosis and rheumatoid arthritis, where they contribute to the inflammatory milieu and tissue damage. The numerous illnesses that might arise if lysosomal membrane homeostasis is compromised highlight the significance of this equilibrium in physiology (Radulovic *et al.*, 2026). Excessive fluid buildup in hypotonic solutions causes the RBC membrane to break, allowing serum proteins and fluids to escape into tissues (Nakka *et al.*, 2025).

Subsequently, the HRBC membrane stabilization technique is used to investigate the extract's anti-inflammatory properties *in vitro*. Anti-inflammatory activity was measured as the percentage of RBC lysis, and NSAIDs were employed as a benchmark. The lysosomal membrane and the HRBC membrane function similarly. If the extract is used to stabilise it, the lysosomal membrane is likewise stabilised (Yesmin *et al.*, 2020).

Furthermore the enzyme cyclooxygenase (COX), which is in charge of converting arachidonic acid into prostaglandins, is inhibited by NSAIDs (Khalil *et al.*, 2024). In a similar vein, non-steroidal anti-inflammatory medications like diclofenac sodium function as lysosomal membrane stabilizers or by blocking lysosomal enzymes (Kherbache *et al.*, 2020).

Ultimately the experiment's findings indicate that the *Cistanche tubulosa* flower extract has a preventive effect against hypotonic conditions. These findings imply that our plant's high phenolic compound content is related to its anti-inflammatory qualities. The main metabolic processes that are thought to underlie flavonoid activity include altering the stability and structure of membranes as well as affecting their permeability. Flavonoids interact with the hydrophobicity, planarity, and polarity of membranes. Flavonoids' structural changes, such as halogenation, hydroxylation, or glycosylation, are associated with a preference for lipid membranes and affect both the degree of membrane fluidity and electrostatic interactions with polar areas (Dudek *et al.*, 2024).

There were no research on the anti-inflammatory properties of *Cistanche tubulosa* flowers extract in the literature review. Moreover, the anti-inflammatory properties of species within the same genus have been the subject of relatively few investigations. And according to (Bougandoura *et al.*, 2016) the activity of the EtOAc extract of the species *C. violacea* is similar to that obtained in our study of the aqueous extract of the flowers of *Cistanche tubulosa*, given that both species were collected in southern Algeria.

II.3. Antioxidant activities

Combining multiple antioxidant assays is crucial for evaluating extracts antioxidant capacity. Antioxidant activity can be attributed to a variety of mechanisms, including scavenging free radicals, chelating redox metals to inhibit the production of OH[•] through Fenton-like reactions, repairing oxidatively damaged biomolecules, and modulating the antioxidant/oxidant enzymatic system (Galano, 2025).

II.3.1. DPPH radical scavenging effect

At the outset the study's findings demonstrate the outstanding scavenging properties of *Cistanche tubulosa* flower aqueous extract against the DPPH radical. In fact, the extract yielded high IC₅₀ values (more than 30.88 µg/ml), so the reference antioxidant BHT is more active than the aqueous extract. The direct scavenging of reactive oxygen species (ROS) is one of the many ways that flavonoids' antioxidant qualities work. Flavonoids aromatic structures contain hydroxyl groups that enable them to counteract free radicals and lessen oxidative stress, a harmful process linked to a number of chronic illnesses.

The polyphenolic structure of flavonoids, which permits powerful interactions with oxidative species through hydrogen bonds and other chemical interactions, is thought to be responsible for these antioxidant properties. But flavonoids' antioxidant properties go beyond just scavenging free radicals. They have been demonstrated to enhance the expression of natural antioxidant enzymes while also modifying cellular antioxidant defense mechanisms (Bas, 2026).

According to the consulted literature, and according (Rahim *et al.*, 2022) findings suggest a synergistic antioxidant effect of *Cistanche phylloepae* extract with decoction, with IC₅₀ values lower than those we found, reflecting a higher efficacy than that of the extract in our study. These differences are due to the difference in the species studied.

II.3.2. Reducing power

The Fe³⁺ reduction test is commonly used to evaluate the antioxidant capacity of diverse biological samples, especially plant extracts. It is based on the reduction of ferric ions (Fe³⁺) to ferrous ions (Fe²⁺) in the presence of antioxidants acting as reducing agents. Reducing substances include organic acids, thiols, flavones, and phenolic chemicals help change Fe³⁺ into Fe²⁺ (Gulcin & Alwasel, 2025). And it illustrates the outcomes of the *Cistanche tubulosa* flower aqueous extract, which shown a potent reduction capacity. Therefore, this extract's polyphenolic constituents, such flavonoids, directly contribute to its efficacy. Previous research on various *Cistanches herba* extracts showed a good reduction power using the FRAP assay (Rahim *et al.*, 2022).

II.3.3. AAPH induced erythrocyte oxidative hemolysis assay

2,2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH)-stimulated red blood cells (RBCs) are a useful system for studying oxidative stress-related pathological states that affect the integrity of human red blood cells and cause multi-organ dysfunction, such as hemolytic anemia, vaso-occlusion, chronic inflammation, and tissue damage, among other cell-based models of oxidative stress (Remigante *et al.*, 2023). Erythrocytes pre-incubated with varying doses of *C. tubulosa* flower extract demonstrated a potent protective effect against erythrocyte lysis caused by AAPH. There was a concentration-dependent increase in this protective effect. The Aq.E exhibited a significant ($p < 0.001$) antihemolytic activity at a concentration of 40 µg/mL with half hemolysis time (HT₅₀) of 68.80 ± 0.72 min . This value is less than those obtained with ascorbic acid at the same concentration (122.30 ± 0.22 min). The effectiveness of *C. tubulosa* flowers extract in the increasing of HT₅₀ could be attributed to its high phenolic and flavonoids contents. However, the findings demonstrated that by preventing AAPH-induced oxidative damage in erythrocytes, flavonoids might shield cell membranes from oxidative damage by free radicals (Zheng *et al.*, 2022).

Interactions between flavonoids and cell membranes are an important focus and may provide insight into their methods of action. The main metabolic processes that are thought to underlie flavonoid activity include scavenging free radicals, altering the stability and structure of membranes, and affecting membrane permeability. Flavonoids' hydrophobicity, planarity, and polarity are some of the factors that affect how they interact with membranes. Flavonoids' structural changes, such as halogenation, hydroxylation, or glycosylation, are associated with an affinity for lipid membranes and influence both the degree of membrane fluidity and electrostatic interactions with polar areas (Dudek *et al.*, 2024). In the literature studied, the AAPH assay was never used to investigate the antioxidant activity of *Cistanche tubulosa* flower extract.

Conclusion and perspectives

Conclusion and perspectives

Medicinal plants continue to be a dependable source of active components with medicinal qualities. Most modern drugs are concentrated versions of plant treatments, especially polyphenols, which are among the most intriguing and researched substances available today. The polyphenols and flavonoids in the aqueous extract of the blooming portion of *Cistanche tubulosa* were first measured in this investigation. The same extract's antioxidant potential was then assessed *in vitro*. Significant levels of total polyphenols and flavonoids were found in the *Cistanche tubulosa* extract, according to quantitative analysis of these compounds using the Folin-Ciocalteu and aluminum trichloride techniques, respectively. *Cistanche tubulosa* flowers aqueous extract has an outstanding antioxidant effect. The extract under investigation showed a strong reducing capacity and modest suppression of the DPPH and AAPH free radicals. The study's findings about the aqueous extract of *Csitanche tubulosa*'s floral parts anti-inflammatory and antioxidant qualities are highly intriguing, but more thorough research is required to comprehend their molecular and cellular processes. These investigations ought to concentrate on separating the bioactive substances from the extracts and assessing how these substances affect signaling pathways, ROS-producing enzymes, antioxidant systems, and the cellular and molecular processes of inflammation.

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تهدف هذه الدراسة الى تقييم النشاط المضاد للالتهاب و المضاد للأكسدة للمستخلص المائي للجزء الزهري لنبته *Cistanche tubulosa* . بلغ مردود المستخلص الناتج عن الغليان حوالي 26% و اظهرت التجارب الكيميائية ان المستخلص يحتوي على كمية ملحوظة من المركبات متعددة الفينول و الفلافونويدات المقدرة بـ $56,74 \pm 1,54$ ميكروغرام مكافئ حمض الغاليك\مغ مستخلص و $10,540 \pm 0,474$ ميكروغرام من مكافئ الكيرسيتين\مغ مستخلص. لدراسة النشاط المضاد للالتهاب للنبته تم اجراء اختبار استقرار الغشاء لكريات الدم الحمراء من دم شخص متبرع سليم ، اظهرت النتائج أن المستخلص يمتلك قدرة عالية على حماية غشاء الكريات الحمراء بنسبة 100 % عند تركيز 3مغ/مل متجاوزاً فعالية الدواء المرجعي Diclofenac المستعمل للمقارنة. كما اظهر المستخلص نشاطاً مضاداً للأكسدة في اختبارات DPPH و FRAP حيث امتلك قدرة فعالة على تثبيط الجذور الحرة والاختزال، وإن كانت أدنى من فعالية مضاد الأكسدة المرجعي المستعمل BHT بقيم IC_{50} تقدر بـ $135,41 \pm 0,95$ و $30,88 \pm 2,16$ مكغ/مل و EC_{50} تقدر بـ $178,86 \pm 4,1$ و $30,56 \pm 0,79$ مكغ/مل على التوالي . بالإضافة الى ذلك، فقد ثبت ان المستخلص يمتلك تأثيراً وقائياً كبيراً ضد تحلل كريات الدم الحمراء الناجم عن AAPH، مع زيادة في زمن نصف انحلال الدم (HT_{50}) بشكل مرتبط بالتركيز. بلغت قيمة HT_{50} المسجلة 68.80 ± 0.72 دقيقة و 122.30 ± 0.22 دقيقة للمستخلص و لحمض الاسكوربيك المرجعي على التوالي بتركيز 40 ميكروغرام/مل. كخلاصة، يمكن اعتبار *Cistanche tubulosa* مصدراً واعداداً لمضادات الالتهاب ومضادات الأكسدة.

الكلمات المفتاحية: مضادات الالتهاب، مضادات الأكسدة، *Cistanche tubulosa*، الإجهاد التأكسدي، المركبات الفينولية.

Résumé

Cette étude vise à évaluer l'activité anti-inflammatoire et antioxydante de l'extrait aqueux de la partie florale de la plante *Cistanche tubulosa*. Le rendement de l'extrait obtenu par décoction s'est élevé à environ 26 % et les analyses chimiques ont montré que l'extrait contenait une quantité notable de composés polyphénoliques et de flavonoïdes, estimée respectivement à $56,74 \pm 1,54$ microgrammes d'équivalent acide gallique/mg d'extrait et $10,540 \pm 0,474$ microgrammes d'équivalent quercétine/mg d'extrait, respectivement. Afin d'étudier l'activité anti-inflammatoire de la plante, le test de stabilité membranaire des globules rouges provenant du sang d'un donneur sain a été réalisé, les résultats ont montré que l'extrait possède une forte capacité à protéger la membrane des globules rouges à 100 % à une concentration de 3 mg/mL, surpassant l'efficacité du médicament de référence, le diclofénac de sodium, utilisé comme anti-inflammatoire de référence. L'extrait a également montré une activité antioxydante lors des tests DPPH et FRAP, où il a démontré une capacité efficace à inhiber les radicaux libres et à réduire ces derniers, bien que cette activité soit inférieure à celle de l'antioxydant de référence utilisé, le BHT, avec des valeurs de IC_{50} $135,41 \pm 0,95$ et $30,88 \pm 2,16$ µg/mL et des valeurs EC_{50} $178,86 \pm 4,17$ et $30,56 \pm 0,79$ µg/mL respectivement. De plus, l'extrait s'est révélé posséder un effet protecteur important contre l'hémolyse induite par l'AAPH, avec une augmentation du temps de demi-vie hémolytique (HT_{50}) proportionnelle à la concentration. Les valeurs de HT_{50} enregistrées étaient respectivement de $68,80 \pm 0,72$ minutes et $122,30 \pm 0,22$ minutes pour l'extrait et l'acide ascorbique de référence, à une concentration de 40 microgrammes/ml. En conclusion, *Cistanche tubulosa* peut être considérée comme une source prometteuse d'agents anti-inflammatoires et antioxydants.

Mots-clés : Anti-inflammatoires, antioxydants, *Cistanche tubulosa*, stress oxydatif, composés phénoliques.

Abstract

This study aims to evaluate the anti-inflammatory and antioxidant activity of the aqueous extract of the flowering parts of the plant *Cistanche tubulosa*. The extraction yield from decoction was approximately 26%, and chemical analyses showed that the extract contains a significant amount of polyphenolic compounds and flavonoids, estimated at 56.74 ± 1.54 micrograms of gallic acid equivalent / mg extract and $10,540 \pm 0.474$ micrograms of quercetin equivalent/mg extract, respectively. To study the plant's anti-inflammatory activity, a red blood cell membrane stability test was conducted using blood from a healthy human donor, The results showed that the extract possessed a high ability to protect red blood cell membranes, with 100% protection at a concentration of 3 mg/mL, surpassing the efficacy of the reference drug diclofenac used as anti-inflammatory standard. The extract also exhibited antioxidant activity in DPPH and FRAP assays, demonstrating an effective ability to inhibit free radicals and reduce oxidized compounds, although its efficacy was lower than that of the reference antioxidant BHT, with IC_{50} 135.41 ± 0.95 and 30.88 ± 2.16 mg/mL and EC_{50} 178.86 ± 4.17 and 30.56 ± 0.79 mg/mL, respectively. In addition, the extract was found to have a significant protective effect against AAPH-induced hemolysis, with a concentration-dependent increase in the half-life of hemolysis (HT_{50}). The recorded HT_{50} values were 68.80 ± 0.72 minutes and 122.30 ± 0.22 minutes for the extract and the ascorbic acid reference, respectively, at a concentration of 40 µg/mL. In conclusion, *Cistanche tubulosa* can be considered a promising source of anti-inflammatory and antioxidant compounds.

Keywords: anti-inflammatory agents, antioxidants, *Cistanche tubulosa*, oxidative stress, phenolic compounds.