الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

Université Ferhat Abbas Sétif 1
Faculté des Sciences de la Nature
et de la vie

جامعة فرحات عباس، سطيف1
كلية علوم الطبيعة والحياة

DEPARTEMENT OF BIOCHEMISTRY

N°……/SNV/**2023**

THESIS

Presented by

Amina Lamouri

For the fulfillment of the requirements for the degree of

DOCTORATE 3rd Cycle

BIOLOGICAL SCIENCES

Special filed: **BIOCHEMISTRY**

TOPIC

Evaluation of antioxidant and anti-inflammatory activities of *Hypericum tomentosum L.* leaves extracts

Presented publically in: 12/21 /2023.

JURY:

President :	**Hamama Bouriche**	**Pr. UFA Setif 1**
Supervisor:	**Dalila Messaoudi**	**MCA. UFA Setif 1**
Co-supervisor:	**Seoussen Kada**	**MCB. UFA Setif 1**
Examiners:	**Selma Houchi**	**MCA. UFA Setif 1**
	Asma Mosbah	**Pr. Univ Costantine**
	Dalila Bencheikh	**MCA. Univ M'sila**

Laboratory of Applied Biochemistry

List of publications

Publication

1. **Lamouri, A.,** Messaoudi, D., kada, S., Bouriche, H., Senator, A. (2022). Antioxidant activity of aqueous extract of leaves from *Hypericum tomentosum*. *Indian Journal of Novel Drug Delivery*, 14 (4), 192-199.

Proceeding

1. **Lamouri, A.**, Messaoudi, D., Kada, S., Harieche M., Meziti, H., Hamama Bouriche H. (2021). Free radical scavenging, ferrous anion chelating and antihemolytic activity of quercetin. Proceeding of International Conference on Food, Agriculture and Animals science. 13- 17 October 2021. Erzurum, Turkey.

Communications

1. **Lamouri, A.**, Messaoudi, D., Kada, S., Harieche, M., Meziti, H., Boussif, A., Bouriche, H. *In vitro* antioxidant properties of rutin. 1st internatinal days of natural and life science (INDNS 2022). 01 and 02 March 2022. Ecole Normale superieur des Enseignants. Ouargla, Algerie. Oral communication.

2. **Lamouri, A.,** Messaoudi, D.,Kada, S., Bouriche, H., Senator, A. β-carotene bleaching and hydroxyl radical scavenging activities of *Hypericum tomentosum*'s aqueous extract. 1er séminaire national sur l'évaluation des activités biologiques des plantes médicinales et docking moléculaire. 22 Mars 2022. Université Batna 2, Algérie. Poster.

3. **Lamouri, A.,** Messaoudi, D., Kada, S., Harieche, M., Meziti, H., Boussif, A., Bouriche, H. Hydroxyl radical and hydrogen peroxide scavenging activities of *Hypericum tomentosum* aqueous extract. 1er seminaire nationale sur la valorization des ressources naturelles et de l'environnement (VNRE 2022). 30 Mars 2021. Université Ferhat Abbas, Sétif 1, Algerie. Poster.

4. **Lamouri, A.,** Messaoudi, D., Kada, S., Bouriche, H., Senator, A. Anti-hemolytic and ferrous ion chelating activities of aqueous extract of *Hypericum tomentosum*. 5th International Conference on Life and Engineering Sciences with the sponsorship of Anand International College of Engineering. May 19-22, 2022. Antalya, Turkey.Oral communication.

5. **Lamouri, A.,** Messaoudi, D., Kada, S., Boussif, A., Meziti, H., Bouriche, H., Senator, A. Inhibition of myeloperoxidase activity by methanolic extract of *Hypericum tomentosum*. 5th International Conference on Life and Engineering Sciences with the sponsorship of Anand International College of Engineering. May 19-22, 2022. Antalya, Turkey.Oral communication.

6. **Lamouri, A.,** Boussif, A., Messaoudi, D., Kada, S., Bouriche, H., Senator, A. The inhibition of *Escherichia coli* killing by human neutrophils pretreated by *Hypericum tomentosum* methanolic extract. International microbiology webinar. June 4th, 2022. Bejaia, Algeria. Oral communication.

7. **Lamouri, A.,** Messaoudi, D., Kada, S., Bouriche, H., Senator, A. Phytochemical screening, DPPH scavenging activity and reducing power of *Hypericum tomentosum* methanolic extract. Seminaire national sur la valorization des bioressources végétales en Algérie. 07 Juin 2022. Université de Khenchla, Algérie. Oral communication.

8. **Lamouri, A.,** Messaoudi, D., Kada, S., Bouriche, H., Senator, A. Hydroxyl radical scavenging and ferrous ion chelating activities of *Hypericum tomentosum* methanolic extract. National Seminar on Phytotherapy and Pharmacognosy (NSPP- 2023). March 14-15, 2023. Setif, Algeria. Poster.

Dedications

First of all, I would like to dedicate this thesis to my dear mother, for her love, generosity, and tenderness, as well as for her sacrifices and prayers. I also dedicate this work to my dad, he also played a big role through different ways of support for me to be here today.

I also dedicate this work to my adorable sisters and brothers, thank you for being there for me and supporting me unconditionally. I'm extremely grateful for all of you.

Furthermore, I would want to express my gratitude to all of my friends who are PhD students or doctors, including; Harieche Mohamed, Koudoune Chahrazed, Laroui Haifa, Abed Lina and Derguine Rania.

Amina

Acknowledgements

I would want to thank Allah, the Almighty, the Most Gracious, and the Most Merciful first and foremost for His blessings on me during my studies and in finishing my thesis.

A special thank you to my supervisor, Dr. Dalila Messaoudi, and my co-supervisor, Dr. Seoussen Kada, whose encouragement, direction, and general expertise in this subject have made this experience for me inspiring. This research would not be possible without their clear instructions and intelligent remarks on every part of this dissertation.

I would like to express my sincere gratitude to Pr. Hamama Bouriche for doing me the honor of presiding my jury and agreeing to evaluate this work and for for all her encouragements.

I am extremely thankful to Dr. Selma Houchi, Dr. Dalila Bencheikh and Asma Mosbah to give the valuable feedback to evaluate and improve the contents of this thesis.

My deep appreciation for Dr. Hicham Meziti and Dr. Abdelali Boussif for helping to realize a part of this work.
I thank Drs. Oguz Cakir and Mustafa Abdullah Yilmaz for their collaboration to realize phytochemical analysis using LC-MS/MS analysis.

I would like to deeply thank Dr. Abdallah kherbacke and Dr. Somia Benbrinis for Their encouragement and guidance which have given me confidence in my research ability.

Finally, I want to sincerely thank and appreciate my family, friends, and everyone else that helped and stood by me while I was working on this project.

الملخص

تهدف هذه الدراسة إلى تقييم الفعالية المضادة للإلتهاب و المضادة للأكسدة للمستخلصين المائي (Aq.E) و الميثانولي (Met.E) لأوراق نبتة *Hypericum tomentosum*. بين التقدير الكمي للمركبات الفينولية أن المستخلص الميثانولي لهذه النبتة يحتوي على أعلى كمية من عديدات الفينول، الفلافونويدات والدباغ مقارنة بالمستخلص المائي. أظهر تحليل HPLC وجود العديد من الفلافونويدات والأحماض الفينولية حيث سجل الكرسترين والإيبيكاتيشين أعلى نسبة من بين الفلافونويدات في حين سجل كل من حمض الكينيك وحمض الكلوروجينيك أعلى تركيز من بين الأحماض الفينولية. تم تقدير النشاطية المضادة للأكسدة للمستخلص المائي و الميثانولي من خلال تحديد أنشطة الكسح ل $DPPH^{\bullet}$، $OH^{\bullet}$ و H_2O_2 ،أظهرت النتائج أن المستخلصين المائي و الميثانولي ثبطا جذور $DPPH^{\bullet}$ بقيم IC_{50} مساوية مساوية ل 9,32 و 5,85 ميكروغرام/ مل على التوالي، كما ثبطا جذور $OH^{\bullet}$ بقيم IC_{50} مساوية ل 338,8 و 377,6 ميكروغرام/ مل على التوالي، وكانت قيم IC_{50} مع H_2O_2 3,13 و2,07 ميكروغرام/ مل على التوالي. من جهة أخرى، أظهر كلا المستخلصين قدرة بالغة على إرجاع الحديد وقدرة على تثبيط أكسدة الليبيدات من خلال التأثير الوقائي الكبير ضد تحلل كريات الدم الحمراء الناجم عن الجذور الحرة وأيضا من خلال تثبيط أكسدة حمض اللينولوييك بنسبة 77,48 % للمستخلص المائي و بنسبة 29,43 % للمستخلص الميثانولي، في حين كانت قدرة المستخلصين على إستخلاب أيونات الحديد ضعيفة. بينت نتائج النشاطية المضادة للإلتهاب أن معالجة الفئران ب 1 مغ/مل من المستخلص المائي و الميثانولي إلى تثبيط معنوي ($p<0,001$) في هجرة الخلايا المتعادلة بنسبة 38,53 % و 47,5 % على الترتيب، كما أدى التطبيق الموضعي لكلا المستخلصين المائي و الميثانولي في نموذج وذمة الأذن الناجمة عن زيت الكروتون عند الفئران إلى تقليل الوذمة بنسبة 68,75 % و 67 % على التوالي، في حين أظهرت معالجة الفئران بتركيز 200 و 400 مغ/ كغ من كلا المستخلصين عن طريق الفم فعالية ضعيفة في تقليل وذمة الرجل المحدثة بالكراجينين. أبدى كلا المستخلصين بنفس التراكيز قدرة على تثبيط الألم المحدث بحمض الأستيك. أثبت كلا المستخلصين نشاط مضاد للالتهاب من خلال اختبارات تقييم تأثيرهما على وظائف الخلايا البيضاء المتعادلة البشرية، حيث أظهرا تثبيطًا كبيرًا و متعلق بالتركيز على نشاط كل من إنزيم الإيلاستاز و إنزيم الميالوبيروكسيداز، كما بين كلا المستخلصين فعالية معتبرة ($p<0,05$) على تثبيط الخلايا المتعادلة على قتل *Escherichia coli* ومع ذلك ، في اختبار البلعمة الخلوية لخلايا خميرة *Candida albicans* ، أبدى المستخلصان اللذان تمت دراستهما تثبيطا طفيفا وغير معنوي لنسبة البلعمة.

الكلمات المفتاحية: مضادات الإلتهاب، مضادات الأكسدة، *Hypericum tomentosum*، الفلافونوييدات، عديدات الفينول.

Abstract

This study aims to evaluate the anti-inflammatory and antioxidant activities of aqueous (Aq.E) and methanolic (Met.E) extracts of *Hypericum tomentosum* leaves. The quantitative assessment of phenolic compounds showed that Met.E extract of this plant contains the highest amount of polyphenols, flavonoids and tannins compared to Aq.E. HPLC analysis showed the presence of many flavonoids and phenolic acids, quercetrin and epicatechin recorded the highest amount among flavonoids, while quinic acid and chlorogenic acid had the highest concentration among the phenolic acids. The antioxidant activity of Aq.E and Met.E was estimated *in vitro* by determining the scavenging activity of $DPPH^{\bullet}$, $OH^{\bullet}$ and H_2O_2. The results show that both Aq.E and Met.E inhibited $DPPH^{\bullet}$ with IC_{50} values of 9.32 and 5.85 µg/mL, respectively and they inhibited $OH^{\bullet}$ with IC_{50} equal to 338.8 and 377.6 µg/mL and the values of IC_{50} with H_2O_2 were 3.13 and 2.07 µg/mL, respectively. Both extracts also showed a great ability to reduce ferric ion and inhibit lipid oxidation, through inhibition of erythrocytes lysis caused by radicals attack and through prevention of linoleic acid oxidation by inhibition levels of 77.48% with Aq.E and 29.43% with Met.E, while the ability of the two extracts to chelate ferrous ions was weak. The results of anti-inflammatory activity demonstrated that treating mice with 1 mg/mL of Aq.E and Met.E extracts significantly (p<0.001) inhibited neutrophils migration by 38.53% and 47.5%, respectively. Topical application of both Aq.E and Met.E to mice, in the model of ear edema induced by croton oil, reduced edema by 68.75% and 67%, respectively. While orally treatment of mice with a concentration of 200 mg/kg and 400 mg/kg of both extracts showed a weak effectiveness in reducing paw edema induced by carrageenan. The same concentrations proved an ability to inhibit pain caused by acetic acid. Both extracts showed anti-inflammatory activity through tests evaluating the effects of the studied extracts on human neutrophils functions. They exerted a big inhibition and dose-dependent manner elastase and myeloperoxidase activities. The two extracts were shown to be able to inhibit significantly (p<0.05) bactericidal activity exerted on *Escherichia coli*. However, in assay of cellular phagocytosis of *Candida albicans* cells by human neutrophils, the studied extracts showed slight and non-significant inhibition of phagocytosis.

Keywords: Anti-inflammatory, Antioxidant, *Hypericum tomentosum*, Flavonoids, Polyphenols.

Résumé

Cette étude vise à évaluer l'activité anti-inflammatoire et anti-oxydante de l'extrait aqueux (E.Aq) et méthanolique (E.Met) des feuilles de la plante *Hypericum tomentosum.* L'évaluation quantitative des composés phénoliques montre que l'E.Met de cette plante contient des quantités plus élevées en polyphénols, flavonoïdes et en tanins par rapport à l'E.Aq. L'analyse HPLC a montré la présence de nombreux flavonoïdes et acides phénoliques, la quercitrine et l'épicatéchine ont enregistré la concentration la plus élevée parmi les flavonoïdes, tandis que l'acide quinique et l'acide chlorogénique existent avec des concentrations les plus élevées parmi les acides phénoliques. L'activité anti-oxydante de E.Aq et E.Met a été estimée *in vitro* par la détermination de l'activité scavenger de $DPPH^{\bullet}$, de l'$OH^{\bullet}$ et de l'H_2O_2. Les résultats ont montrés que l'E.Aq et l'E.Met ont inhibé le $DPPH^{\bullet}$ avec les valeurs d'IC_{50} de l'ordre de 9,32 et 5,85 µg/mL, respectivement. Ils ont inhibé aussi le $OH^{\bullet}$ avec des IC_{50} de 338,8 et 377,6 µg/mL, respectivement. Les valeurs des IC_{50} avec l'H_2O_2 sont égales à 3,13 et 2,07 µg/mL, respectivement. Les deux extraits ont également montré une forte capacité à réduire le fer ferrique et à inhiber l'oxydation des lipides, à travers la prévention contre la lyse des globules rouges causée par l'attaque radicalaire et via l'empêchement de l'oxydation de l'acide linoléique avec des niveaux d'inhibition de 77,48 % avec l'E.Aq et de 29,43 % avec l'E.Met. Bien que les deux extraits aient un faible effet chélateur des ions ferreux. Les résultats de l'activité ant-iflammatoire montrent que le traitement des souris avec 1 mg/mL de l'E.Aq et l'E.Met inhibe significativement (p<0,001) la migration des neutrophiles avec des taux de 38,53 % et 47,5 %, respectivement. L'application topique de l'E.Aq et l'E.Met chez les souris dans le modèle de l'œdème de l'oreille induit par l'huile de croton a réduit l'oedème avec des taux d'inhibition de 68,75 % et 67 %, respectivement. Tandis que le traitement par voie orale des deux extraits à 200 mg/kg et 400 mg/kg chez les souris ait montré un faible effet sur la réduction de l'œdème de la patte causé par la carragénane, les mêmes concentrations de l'extrait aqueux et méthanolique ont exercé un effet inhibiteur sur la douleur causée par l'acide acétique. En évaluant l'effet des extraits étudiés sur les fonctions des neutrophiles humains, les deux extraits ont montré une grande inhibition dose-dépendante de l'activité de l'élastase et de la myéloperoxydase. Ces deux extraits se sont révélés capables d'inhiber significativement (p<0,05) l'activité bactéricidie exercé sur *Escherichia coli*. Cependant, dans le test de la phagocytose de la levure *Candida albicans*, les extraits étudiés ont montré une inhibition légère et non significative de la phagocytose.

Mots clés: Anti-inflammatoire, Antioxydant, flavonoïdes, *Hypericum tomentosum*, polyphenols.

List of figures

List of tables

List of abbreviations

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

ABTS•: 2,2'- Azino-bis (3 -ethylbenzothiazoline -6- sulfonic acid)

AP-1: Activator Protein-1

Aq.E: Aqueous extract

ATP: Adenosine triphosphate

BHT: Butylatedhydroxytoluene

CAT: Catalase

CB: Cytochalasin B

CE: Catechin Equivalent

COX: Cyclo-oxygenase

DAMPs: Damage-Associated Molecular Patterns

DMSO: Dimethyl sulfoxide

DPPH•: 2,2'-diphenyle-1-picrylhydrazyl

EC_{50}: Concentration corresponding to 50% of effectiveness

EDTA: Ethylenediaminetetraacetic acid

FI*:* Fragment ions.

fMLP: Formyl-Methionyl-Leucyl-Phenylalanine

GAE: Gallic acid equivalent

GPx: Glutathione peroxidase

GR: Glutathione reductase

GSH: Glutathione

GSSG: Glutathione disulfide

GST: Glutathione S-transferase

HBSS1: Hanks' Balanced Salt Solution without Ca^{2+} and Mg^{2+}

HBSS2: Hanks' Balanced Salt Solution with Ca^{2+} and Mg^{2+}

HEPES: 4- (2-hydroxyethyl)-1-piperazineethanesulfonic acid

HT_{50}: Half- time hemolysis

HTAB: Hexadecyltrimethylammonium bromide

IC_{50}: Concentration corresponding to 50 % of inhibition

ICAM-1: Inter Cellular Adhesion Molecule

IFN: Interferon

IL: Intrleukin

LC- MS/MS: Liquid chromatography coupled with mass spectrometry

LFA-1: Leukocyte Function-associated Antigen-1

Met.E: Methanolic extract

MI: Molecular ions of the standard analytes

MPO: Myeloperoxidase

NF-κB: Nuclear Factor kappa-B

NK: Natural Killer

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

PAMPs: Pathogen-Associated Molecular Patterns

PBS: Phosphate buffer saline

PMNs: Polymorphonuclear Neutrophils

PRR: Pettern Recognition Receptor

QE: Quercetin Equivalent

R.T: Retention time

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

SD: Standard deviation

SEM: Standard error means

SOD: Superoxide dismutase

TMB: 3, 3`, 5, 5`-tetramethylbenzidine

TNF: Tumor Necrosis Factor

OECD: Organization for economic co-operation and development

UHPLC: Ultrahigh performance liquid chromatograph

VCAM-1: Vascular Cell Adhesion Molecule-1

VLA-4: Very Late activation Antigen-4

List of contents

EXPERIMENTAL PART

MATERIALS AND METHODS

RESULTS AND DISCUSSION

INTRODUCTION

Introduction

Inflammation is thought of as a homeostatic mechanism that is brought on in tissue by injury or infection in order to remove the specific pathogen or to repair the tissue. It consists of the production of numerous inflammatory chemicals, which are typically brought on by the activation of innate immune systems. In some cases, inflammation that extends from acute to chronic can be harmful to tissue and cause cell malfunction or death. (Vezzani et *al.*, 2013).

The treatment of inflammation is usually based on the intake of non-steroidal anti-inflammatory drugs and glucocorticoids. All these anti-inflammatory drugs, regardless of the route of administration, have a risk of gastrointestinal and renal toxicity. Herbal medicine can be used as a substitute with favorable pharmacological characteristics. With low toxicity and side effects, it is cheap and effective in treating several inflammatory diseases. In addition, most countries (80%) in the world use medicinal plants for health purposes and pain treatment (Miara et *al.*, 2019).

On the other hand, the uncontrolled production of reactive oxygen and nitrogen species cause or maintain inflammatory processes. The neutralization of these species by antioxidants, especially of natural origin, can limit the damage to biomolecules (DNA, proteins, lipids and sugars) (Song and Zou, 2015).

Algeria presents a taxonomic biodiversity of plants given its privileged biogeographical position and its extent between the Mediterranean and sub-Saharan Africa. Its richness makes the study of the Algerian flora of enormous scientific interest for researchers.

Therefore, the use of medicinal plants is a good alternative for the search for new therapeutic molecules.

In this context fits this present work whose main objective is to evaluate the anti-inflammatory and antioxidant properties of a medicinal plant commonly used in traditional medicine: *Hypericum tomentosum*

In this study, four aspects are studied.

- The first part is phytochemical study based mainly on the extraction, quantification and identification of phenolic compounds.
- The second part is devoted to the evaluation of the antioxidant activity by several *in vitro* tests (free radical scavenging, hydrogen peroxide scavenging, reducing power, ferrous ion chelating, anti-hemolytic and β-carotene bleaching activities).

- The third part is devoted to evaluating the toxicity of the plant extracts studied, with the aim of defining the toxic threshold and the range of harmlessness of these extracts.
- In the fourth part, the anti-inflammatory activities (*in vivo* and *in vitro*) were studied using several models including human neutrophils elastase and myeloperoxidase activities, anti-bactericidal activity, phagocytosis test, ear edema induced by croton oil, paw edema induced by carrageenan, air pouch and analgesic test.

LITTERATURE REVIEW

Inflammation

Inflammation is the body's complex response to chemical and/or physical aggression (Germolec et *al.*, 2018). It's characterized by the activation of immune and non-immune cells which release mediators like vasoactive amines, eicosanoids and pro-inflammatory cytokines. These mediators are engaged in the inflammatory process by preventing tissue damage and restoring tissue integrity (Abdulkhaleq et *al.*, 2018; Furman et *al.*, 2019).

Inflammation can be acute or chronic. The first mainly involves tissue repair. However, it may develop into chronic inflammation, which is related to many diseases (Chen et *al.*, 2018).

I. 1. Acute inflammation

Acute inflammation is a rapid, dynamic and reversible process leading to its resolution. This physiological response is defined by four clinical signs heat pain, redness and swelling. These symptoms are linked to the effects of the various inflammatory agents present at the site of the aggression (Noack and Kolopp-Sarda, 2018). The acute inflammatory response occurs in three phases.

I. 1. 1. Vascular phase

It occurs as a result of interactions between PAMPs (Pathogen-Associated Molecular Patterns), molecules from an infectious organism, and DAMPs (Damage-Associated Molecular Patterns), which are molecules released by damaged cells of the host, with PRR (Pettern Recognition receptor) expressed by innate immunity cells (**figure 1**), these sentinel cells react rapidly by secreting pro-inflammatory cytokines (Varela et *al.*, 2018). The coagulation cascade is activated and results in the formation of fibrin which forms clots in cooperation with activated platelets (Nasimuzzaman and Malik, 2019).

During this phase, several inflammatory mediators are released: lipid mediators (prostaglandin, leukotriene), vasoactive factors (histamine and serotonin) (Kim, 2016), kinins and complement proteins (Lopatko Fagerström et *al.*, 2019) leading to: vasodilation responsible for redness and heat (Varela et *al.*, 2018), vasodilation is accompanied by an increase in vascular permeability and consequently plasma exudation and thus the formation of edema (Sansbury and Spite, 2016), cell migration, and activation of local nociceptors in damaged tissue, inducing the sensation of pain (Kumar and Jain, 2014).

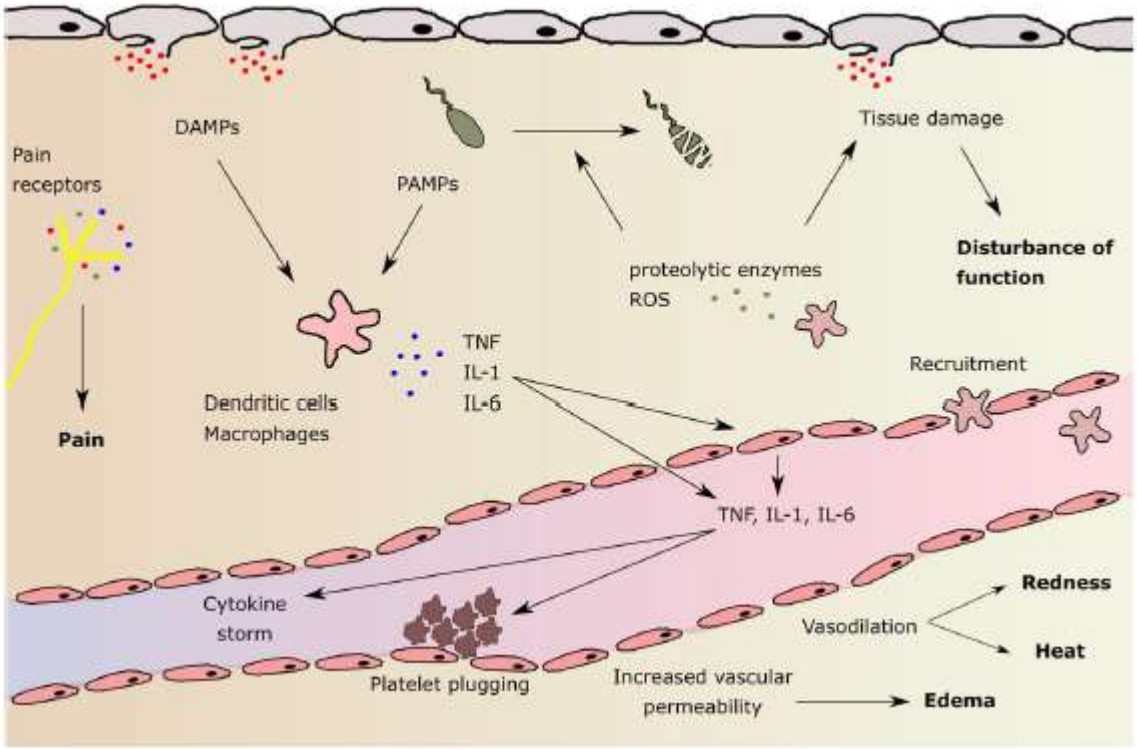

Figure 1. Inflammation initiation and clinical symptoms (Varela et *al.*, 2018).

I. 1. 2. Cellular phase

It is the recruitment of blood leukocytes to damaged tissues, the first cells attracted to the damaged site are neutrophils followed by monocytes and then the migration of lymphocytes (Chen et *al.*, 2018). The cascade of this migration initiates by a weak interaction between the endothelial cells, activated by the inflammatory mediators, and the leukocytes this interaction is ensured by the selectins P and E and their glucosylated ligands (**figure 2**) those which lead to the rolling of the leukocytes. This rolling leads to the interaction of leukocytes with chemokines retained on the surface of endothelial cells, which leads to the activation of leukocyte integrins such as LFA-1 (Leukocyte Function-associated Antigen-1) and VLA-4 (Very Late activation Antigen-4). LFA-1 binds with ICAM-1 (Inter Cellular Adhesion Molecule) leading to firm adhesion of neutrophils, while the interaction between VLA-4 and VCAM-1 (Vascular Cell Adhesion Molecule-1) is important in the binding of monocytes with the endothelium (Kourtzelis et *al.*, 2017) and then the migration through the endothelial tissue, usually at the anchorage point where two cells connect through the membrane (Castan et *al.*, 2016).

Once leukocytes reach the inflammatory focus, neutrophils defend against invading pathogen and degrade it in a phagolysosome and they release reactive oxygen species (ROS) and lytic enzymes (Sansbury and Spite, 2016; Germolec et *al.*, 2018).

The second wave of cells consists of monocytes, which reach the focus of inflammation, where they transform into macrophages, and play an important role in phagocytosis, antigen presentation and immune system regulation through the production of cytokines and growth factors (Chen et *al.*, 2018).

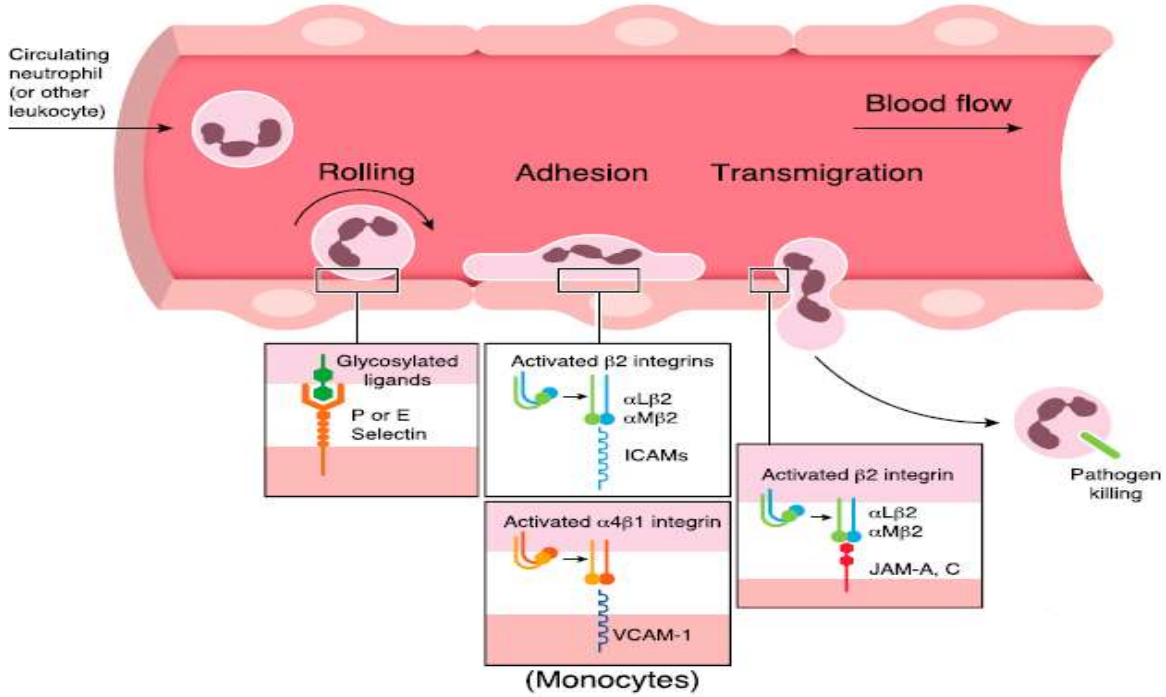

Figure 2. Leukocyte diapedesis (Kourtzelis et *al.*, 2017).

I. 1. 3. Resolution phase

Under physiological conditions, the inflammatory reaction is followed by the resolution phase. This stage is considered to be an active process involving a variety of cellular and molecular mechanisms, allowing the restoration of homeostasis and the restoration of the integrity of the damaged tissues (Barnig, 2016).

The resolution of inflammation is a process of fine regulation. At this stage, the gradient of chemokines is diluted with time, the infiltration of neutrophils to tissues is reduced or stopped, and these neutrophils are apoptotic. Macrophages are reprogrammed and transformed from pro-inflammatory phenotype to anti-inflammatory phenotype macrophages M2 which are a powerful phagocytes of apoptotic cell fragments, which play a role in tissue repair (Chen et *al.*, 2018).

Endothelial repair is ensured by endothelial cells (neoangiogenesis) and fibroblasts (collagen) under the effect of cytokines secreted by macrophages (Noack and Kolopp-Sarda, 2018).

I. 2. Chronic inflammation

It is a maladaptive, long-term and dysfunctional response involving persistent inflammation and tissue destruction (Bernstein et *al.*, 2018). It may last for a long time and lead to the loss of inflammatory regulation process, resulting in irreparable tissue damage and many diseases.

I. 3. The effectors of inflammation

I. 3. 1. Cellular effectors

The inflammatory process involves different types of cells, which are both circulating cells that migrate to the interstitial tissue or cells of the inflammatory focus.

Polymorphonuclear neutrophils, neutrophils, or polymorphonuclear neutrophils (PMNs) are cells of the innate immune system that play a very important role in defenses against pathogens. They are the most abundant leukocytes in the blood stream, and their numbers increase very sharply and very rapidly during infections (Gillis and Reber, 2018). These cells are the first to mediate acute inflammation. Their granules include several enzymes, peptides and proteins, they are able to releasing ROS, PMNs migrate from the blood to the damaged site following vasodilation and inhance vascular permeability (Germolec et *al.*, 2018).

Monocytes/ macrophage, during inflammation, circulating monocytes leave the blood stream and migrate to the tissue and they differentiate into macrophages under the effect of growth factors and pro-inflammatory cytokines (Shapouri-Moghaddam et *al.*, 2018).

Eosinophils, are the predominant inflammatory cells during parasitic infection. They are recruited to the inflammatory site under the influence of number of factors including interleukin (IL)-5, IL-2, IL-16, histamine and certain complement proteins (Germolec et *al.*, 2018).

Mast cells, are resident cells in connective tissue. They initiate the inflammatory response via the production of pro-inflammatory mediators: histamine, prostaglandin, leukotriene and proteases (Chen et *al.*, 2018).

Endothelial cell, whose function goes beyond a structural barrier that separates the blood from the outer cavity to the endocrine organ secretes vasoactive vasodilating or vasoconstricting agents (Maruhashi et *al.*, 2019).

Platelets, circulating anucleated cells rapidly recruited to the damaged site and activated locally. The primary function of platelets is the formation of clots by subsequently inhibiting plasma exudation. They work in concert with the coagulation system proteins. Platelets are involved in the inflammatory process via the production of ROS and other mediators such as heparin and serotonin (Germolec et *al.*, 2018).

Lymphocytes, a family that includes B cells, T cells and natural killer (NK) cells. They arise from a common lymphoid progenitor. B and T lymphocytes are involved in the humoral and cell-mediated adaptive immune response. While NKs are involved in innate immunity (Jung et *al.*, 2019).

Fibroblasts, resident cells in all tissues of the body. They are involved in the initiation of inflammation through antigen presentation (Desai et *al.*, 2018), but also in the restoration of tissue integrity and homeostasis (Mescher, 2017).

Basophils, polymorphonuclear basophils form a rare myeloid population, representing less than 1% of blood leukocytes. Activation of polymorphonuclear basophils leading to the establishment of an inflammatory response by the release of preformed pro-inflammatory mediator such as histamine or neoformed such as derivatives of arachidonic acid after the action of phospholipases A2 and C, leading to the production of leukotrienes, prostaglandins and platelet activating factor (Rostan et *al.*, 2014).

I. 3. 2. Soluble effectors

The presence of circulating pathogens leads to the activation of inflammatory cells which produce several types of pro-inflammatory mediators responsible for triggering and/or continuing the inflammatory reaction.

Vasoactive amines and peptides, histamine and serotonin are released following degranulation of mast cells, basophils and platelets, it increases vasodilation and vascular permeability. While substance P is secreted by sensory neurons and induces mast cell degranulation (Kim, 2016).

Eicosanoids, arachidonic acid is a major component of the phospholipid membrane, it represents a substrate of inflammatory mediators called eicosanoids. The lipo-oxygenase enzyme acts on this acid by producing leukotriene which stimulates the infiltration of leukocytes and under the effect of cyclo-oxygenase there will be the production of prostaglandin and thromboxane. The latter induces platelet aggregation. While prostaglandin is a vasodilating agent and increases vascular permeability (Abdulkhaleq et *al.*, 2018).

Kinins, are peptides released by kininogen. During the vascular phase of inflammation, kinins are activated and induce vascular permeability and neutrophil recruitment (Lopatko Fagerström et *al.*, 2019).

Complement, is activated during the vascular phase of inflammation, it is involved in the natural defense of the host but also in the adaptive response, the activation of complement reinforces the inflammatory response by the induction of opsonization, the chemotaxis and anaphylaxis (Lopatko Fagerström et *al.*, 2019).

Coagulation system, of which thrombin and fibrin are part which induce the secretion of chemokines and pro-inflammatory cytokine by leukocytes and platelets and also change the adhesive properties of leukocytes by increasing the expression of selectin P and CD40 ligand (Nasimuzzaman and Malik, 2019).

Platelet activating factor, mediator synthesized from membrane phospholipids by activation of phospholipase A2, by many inflammatory cells. It increases vascular permeability and platelet aggregation, stimulates the attraction of leukocytes and their adhesion to the endothelium (Rousselet and Vignaud, 2005).

Cytokines and chemokines, cytokines are mediators involved in the intercellular communication of the immune system and whose biological activity manifests itself at very low concentrations by binding to specific high-affinity membrane receptors. Their role is to induce, control or inhibit the intensity and duration of the immune response (Joffre and Layé, 2016). The main pro-inflammatory cytokines described are tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-1 and IL-6 (Gupta et *al.*, 2020). During the innate inflammatory response, phagocytic and NK cells release cytokines. However, during an adaptive inflammatory response, they are secreted mainly by lymphocytes and antigen-presenting cells (Germolec et *al.*, 2018).

I. 4. Inflammation and pathologies

Numerous studies indicate that chronic inflammation contributes to the development of several diseases (**figure 3**) such as Alzheimer's disease and osteoporosis (Park et *al.*, 2014), arthritis, asthma, atherosclerosis, aging, diabetes, autoimmune and kidney diseases (Germolec et *al.*, 2018; Furman et *al.*, 2019). It also plays a role in depression in some people, epidemiological studies have demonstrated that high levels of circulating pro-inflammatory cytokines have been linked to depression (Joffre and Layé, 2016).

The chronicity of the inflammation also increases the risk of cancer. It causes carcinogenesis by causing a genetic or epigenetic mutation leading to the initiation of cancer,

inflammatory cytokines and chemokines facilitate the occurrence, proliferation of cancer cells and also angiogenesis (X. Zhang et *al.*, 2017).

In addition, high quantities of thrombin and fibrinogen during inflammation change the clot's structure, creating a denser fibrin network that is more resistant to fibrinolysis and is linked to an increased risk of cardiovascular disease (Picard et *al.*, 2017).

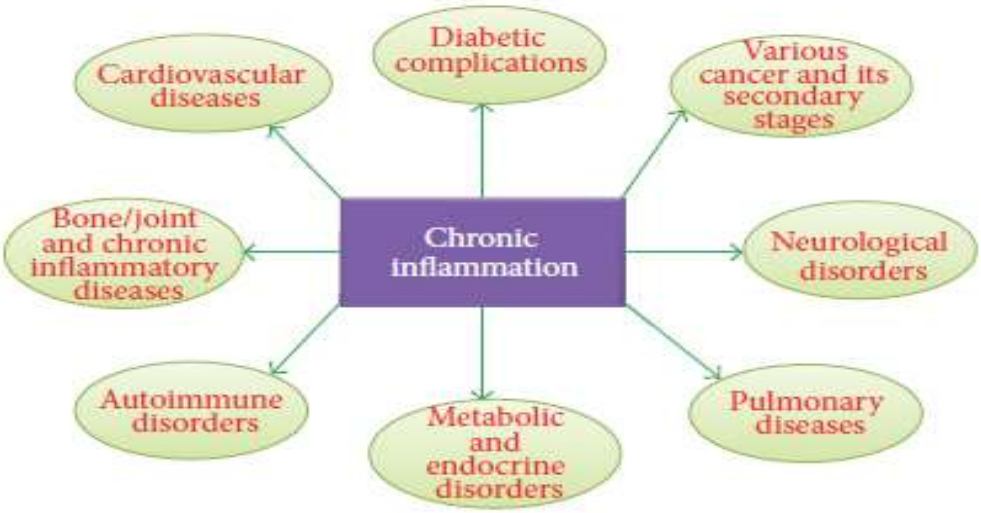

Figure 3. Inflammation related diseases (Arulselvan et *al.*, 2016).

I. 5. Inflammation treatment

I. 5. 1. Non-steroidal anti-inflammatory

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used therapeutic categories in the world, whether in the context of medical prescriptions or self-medication. These drugs have analgesic and antipyretic effects related to anti-inflammatory effects. Non-steroidal anti-inflammatory drugs inhibit the recruitment of neutrophils at inflammatory sites and impair their intrinsic function (Voiriot et *al.*, 2018). This class blocks the expression of cyclooxygenase in cell membranes (**figure 4**), which is essential in the biosynthesis of several prostaglandins from arachidonic acid. There are at least two isoforms of cyclo-oxygenase: cyclo-oxygenase (COX)-1 and COX-2 (Monteiro and Steagall, 2019). COX-1 is constitutive and is involved in tissue hemostasis, while COX-2 is inducible by pro-inflammatory and infectious agents (Nokhbehsaim et *al.*, 2020).Their therapeutic benefit is however limited by the occurrence of potentially serious adverse effects, mainly renal but also digestive (Boussouf et *al.*, 2017; Patil and Patil, 2017).

I. 5. 2. Glucocorticoids drugs

Glucocorticoids constitute a vast family of drugs derived from cortisol. They have anti-inflammatory and immunosuppressive properties (Monteiro and Steagall, 2019) and they are widely used to treat inflammatory and autoimmune diseases (Liberman et *al.*, 2018).

Glucocorticoids prevent the activation of phospholipase, by blocking the prostaglandin pathway (Monteiro and Steagall, 2019) (**figure 4**) and they act on transcription factors such as NF-κB (nuclear factor kappa-B) and AP-1 (activator protein-1) by repression subsequently inhibiting the production of pro-inflammatory cytokines, ROS, expression of cytokine receptors, molecular adhesion and chemotaxis of proteins involved in inflammation (Liberman et *al.*, 2018).

Although glucocorticoids represent the most effective treatment for inflammatory diseases, prolonged administration of these drugs leads to osteoporosis, cataracts, weight gain (Strehl et *al.*, 2011) and also causes endocrine, cardiovascular and neuropsychiatric disorders (Bernstein et *al.*, 2018).

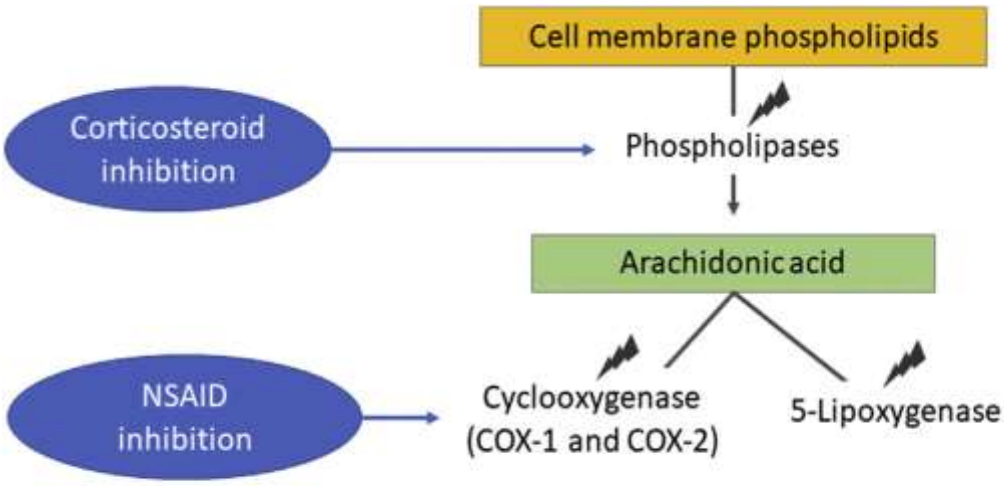

Figure 4. Site of inhibition of cyclo-oxygenases by NSAIDs and site of inhibition of phospholipases by glucocorticoids (Monteiro and Steagall, 2019).

I. 5. 3. Biotherapy

Biotherapy or biological treatment has developed considerably in recent years around the world. This is a varied field of procedures including immunotherapies, cell and gene therapies. These consist respectively of introducing modified cells into the body or acting on the patient's genes. Many agents are used including anti-TNF, anti-IL-1, anti CD20 (Ka et *al.*, 2019).

Although this type of therapy is targeted and presents encouraging results, it is however likely to induce multiple, sometimes severe side effects and these can affect the respiratory system at all levels (Bonniaud et *al.*, 2018).

I. 5. 4. Anti-inflammatory effect of plant source

Phytotherapy can be imposed as an alternative with fewer side effects. The diversity of the plant world and its molecular richness constitute an important source of bioactive molecules of natural origin. Among these bioactive molecules polyphenols, sterols, alkaloids, coumarins, terpenes, saponins, etc (Shahidi and Ambigaipalan, 2015). These active substances can act at several levels of the inflammatory reaction by regulating the activity of certain inflammatory cells such as macrophages, neutrophils and NK cells and by modulating the expression of mediators and pro-inflammatory molecules like COX, nitric oxide synthase, TNFα, IL-1, IL-10 and other cytokines (Patil and Patil, 2017). They are also capable to inhibit ROS production ROS and molecules adhesion expression (Epa et *al.*, 2018; Dramane et *al.*, 2019). In this context, several examples of plants can be cited:

- ✓ *Curcuma longa* (turmeric) contains a yellow pigment called curcumin, a polyphenol that inhibits the transcription of NF-κβ in charge of the production of pro-inflammatory cytokines and downregulates inflammatory enzymes like COX-2 (Bernstein et *al.*, 2018).
- ✓ *Capparis spinosa* is a plant widely used in the Mediterranean region for the relief of arthritis pain. Kernouf et *al.* (2018) reported that the methanolic extract of the flower buds of this plant have an anti-inflammatory effect, due to the richness of the plant by bioactive molecules.
- ✓ Ginger, which has been used in traditional medicine for thousands of years, contains a substance gingerol. This molecule can inhibit the production of nitric oxide and the expression of TNF-α, IL-1, IL-6 and prostaglandin (Liang et *al.*, 2018).

II. Oxidative stress

An imbalance between the oxidant/antioxidant system which results from the excessive production of reactive oxygen and nitrogen species and/or the deficiency in the antioxidant system resulting in oxidative stress (Nemmiche, 2016;Dramane et *al.*, 2019).

II. 1. Free radicals and reactive oxygen and nitrogen species

Free radical is a highly reactive atom or molecule with one or more unpaired electrons in their outer orbital (Liguori et *al.*, 2018). They are very unstable and very reactive chemical substances with a very short lifespan (Yaribeygi et *al.*, 2019).

The word "ROS" refers to compounds that include one or more activated oxygen atoms whether in the form of radical species: hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), alkoxyl ($RO^{\bullet}$) and peroxyl radical ($RO_2^{\bullet}$) or not radical compounds, singlet oxygen ($^1O_2$), hypochlorite ion (ClO^-) hydrogen peroxide (H_2O_2) and organic hydroperoxide (ROOH) (**table 1**) (Zulaikhah, 2017).

Nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxynitrite ($ONOO^-$) and nitroxyl anion (NO^-) are reactive nitrogen species (RNS). More recently RNS have been defined as a subgroup of oxidants derived from nitric oxide (Saddhe et *al.*, 2019) (**table 1**).

Table 1. ROS and RNS (Butterfield and Halliwell, 2019).

	ROS	**RNS**
Radical species	Superoxide ($O_2^{\bullet-}$) Hydroxyl ($OH^{\bullet}$) Peroxyl ($RO_2^{\bullet}$) Alkoxyl ($RO^{\bullet}$)	Nitric oxid ($NO^{\bullet}$) Nitrogen dioxide ($NO_2^{\bullet}$) Nitrate ($NO_3^{\bullet}$)
Not radical species	Hydrogen peroxyde (H_2O_2) Hydroperoxyde (ROOH) Hypochlorous acid (HOCl) Singlet oxygen ($^1O_2$)	Nitrous Acid (HNO_2) Nitrosyl anion (NO^-) Nitrosyl cation (NO^+) Peroxynitrite ($ONOO^-$) Peroxynitrous acid (ONOOH)

II. 2. Generation of reactive oxygen species

Oxygen (or dioxygen, O_2) is essential for the production of energy by many forms of life (animals, plants, bacteria). This production of energy in the form of adenosine triphosphate (ATP) called oxidative phosphorylation takes place in particular via electron transport chains present in the inner membrane of the mitochondria. While about 2% of the oxygen consumed at the mitochondrial level is transformed into superoxide radicals during the first electronic oxygen reduction (Migdal and Serres, 2011). The high reactivity of the superoxide anion can convert it to a relatively stable form that is hydrogen peroxide under the effect of an enzyme, the superoxide dismutase (**figure 5**). Nitric oxide and the superoxide anion can also interact to form peroxynitrite. According to the Fenton reaction, hydrogen peroxide can also break down in the presence of reduced metal ions, such as ferrous iron, to produce the hydroxyl radical, a highly reactive species with a very short half-life (Song and Zou, 2015).

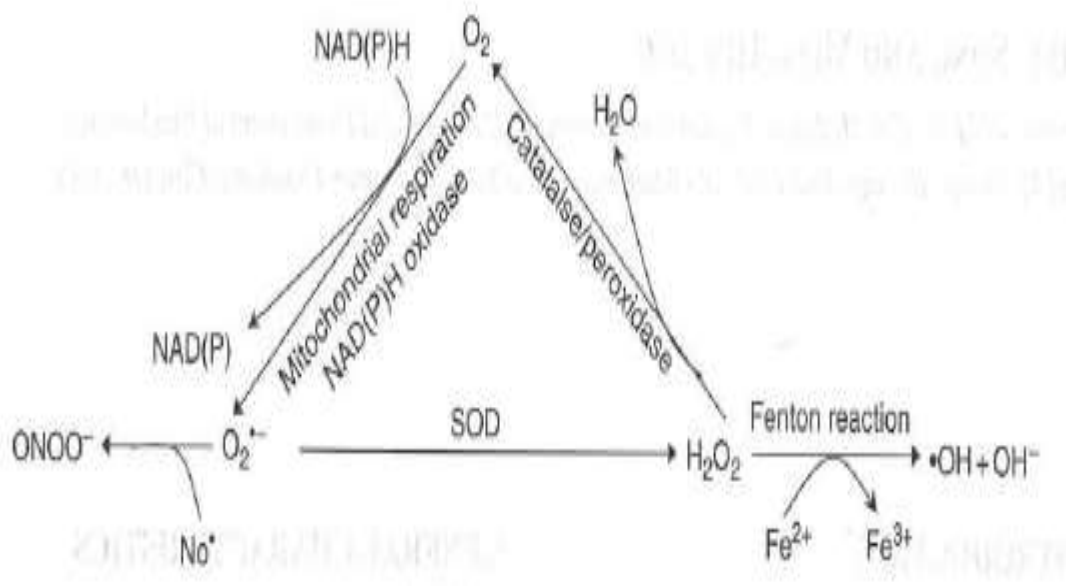

Figure 5. ROS generation (Song and Zou, 2015).

II. 3. Sources of reactive oxygen and nitrogen species

Cellular ROS are generated by both exogenous and endogenous sources.

II. 3. 1. Endogenous source

Mitochondria

The mitochondria generate energy for the cell in the form of ATP via oxidative phosphorylation. The mitochondrial respiratory chain is composed of four protein complexes from I to IV **(figure 6**) and is considered the main endogenous source of ROS generation due to electron leakage causing the reduction of O_2 to $O_2^{\bullet-}$ (Song and Zou, 2015; Yaribeygi et *al.*, 2019).

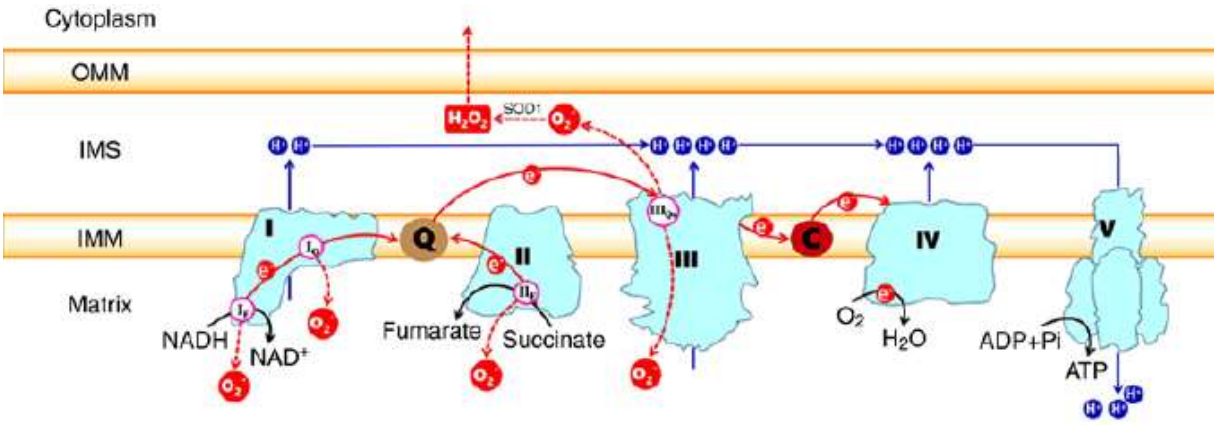

Figure 6. ROS source electron leak within the electron transport chain (OMM, outer mitochondrial membrane, IMS, intermembrane space; IMM, inner mitochondrial membrane; Q, ubiquinone; C, cytochrome) (Zhao et *al.*, 2020).

NAD(P)H oxidases

NAD(P)H oxidaseis an enzyme that catalyzes the one-electron reduction of O_2 using NADPH or NADH as an electron donor:

$$NAD(P)H + 2O_2 \longrightarrow NAD(P)^+ + H^+ + 2\,O_2^{\bullet-}$$

NAD(P)H oxidase is localized in the cytoplasmic membrane. It was initially studied in phagocytic cells where it plays a key role in defense against pathogens, but it also exists in all other non-phagocytic cells where it participates in cell signaling (Migdal and Serres, 2011; Song and Zou, 2015).

Xanthine oxidase

Xanthine oxidase is an enzyme released by the liver and it can bind to the endothelial cell surface. Xanthine oxidase transforms hypoxantine into xanthine and xanthine into uric acid these reactions are accompanied by the release of $O_2^{\bullet-}$ or H_2O_2 the latter can results in the formation of $ONOO^-$ or $OH^{\bullet}$ (Schmidt et *al.*, 2019).

Nitric oxide synthase

Nitric oxide is a radical species synthesized by the three isoforms of nitric oxide synthase nitric oxide synthase: endothelial, neuronal and inducible where L-arginine is converted into citruline and $NO^{\bullet}$ in the presence of oxygen (Song and Zou, 2015; Zhao et *al.*, 2020).

Cytochrome P450

Cytochrome P450 is an essential enzyme involved in the biotransformation of drugs, xenobiotics and also endogenous molecules. During catalytic cycles of cytochrome P450 ROS can generate (Veith and Moorthy, 2018).

II. 3. 2. Exogenous sources

The environment has an impact on the ROS generation. Exposure to ultraviolet itself, therefore to the light rays of the sun, is an activator of the enzyme nitric oxide synthase for the production of nitric oxide (Jagoda and Dixon, 2020).

Xenobiotics such as tobacco, alcohol, drugs, industrial solvents and pesticides participate in the genesis of free radicals (Drzeżdżon et *al.*, 2018; Liguori et *al.*, 2018).

In addition, heavy metals such as cadmium, mercury and cobalt induce the formation ROS (Nemmiche, 2016; Gupta et *al.*, 2020;).

II. 4. Target of reactive oxygen species

Despite ROS play a physiological role in the control of gene expression, cell cycle, apoptosis (Yaribeygi et *al.*, 2019), cell signaling, neurotransmission, muscle relaxation, platelet aggregation and phagocytosis (Santos et *al.*, 2020), their high concentrations are able to attack the macromolecules that make up cells such as lipids, proteins and DNA. Damage to these molecules inevitably leads to cell death.

II. 4. 1. Lipids

The polyunsaturated fatty acids of membrane phospholipids are very sensitive to oxidation by ROS (Song and Zou, 2015). The process of lipid oxidation occurs in three stages: initiation, propagation and termination. The first necessary step is the abstraction of a hydrogen atom from an unsaturated fatty acid molecule RH to form the alkyl radical $R^{\bullet}$. This unstable fatty acid radical reacts rapidly with O_2 to form a $ROO^{\bullet}$ peroxyl radical. There is then transfer of a hydrogen atom by another molecule of fatty acid to form hydroperoxides ROOH this is the propagation step. The oxidized fatty acid turns into an alkyl radical and another cycle begins again (El-Beltagi and Mohamed, 2013).

The interaction of two free radicals and creation of a stable product or the reaction of the radical with an antioxidant molecule like vitamin E can stop the chain reaction (termination phase). (Shahidi and Ambigaipalan, 2015; Cederbaum, 2017).

Lipid peroxidation leads to the formation of highly reactive dialdehydes like manoldialdihyde and 4-hydroxynonenal which in turn leads to changes in membrane fluidity and permeability (Z. Zhang et *al.*, 2017).

II. 4. 2. Proteins

Because of their abundance in the body, proteins are an important target of ROS, which can oxidize proteins by different processes such a modification of this key organic component of life leads to a loss of their function (Demidchik, 2015).

Basic (arginine, histidine, lysine), sulfur (methionine, cysteine) or aromatic (phenylalanine, tryptophan, tyrosine) amino acids are particularly sensitive to oxidation, in particular by addition of carbonyl groups. These carbonyl groups can react with non-oxidized amine functions of lysine to form imine bonds (-HC N-). The oxidation of cysteines leads to the formation of disulphide bridges. The oxidation of two nearby tyrosines can also lead to the formation ofdityrosine, all of these processes can lead to protein aggregation (Durand et *al.*, 2013).

II. 4. 3. Nucleic acids

Nuclear and mitochondrial DNA can also be altered by ROS, nucleic acids are particularly sensitive to the hydroxyl radical. The attack is essentially carried out at the level of the constituent bases of the nucleotides, whether purine or pyrimidine (Baudin, 2020) and induces the modification of the nitrogenous bases, for example the modification of the guanine base to the 8 oxoguanine (**figure 7**). ROS can react with oncogenic or tumor suppressor genes, induce epigenitic alterations and single-strand and double-strand DNA breakage (Li et *al.*, 2018). All these alterations can lead to cell death and aging.

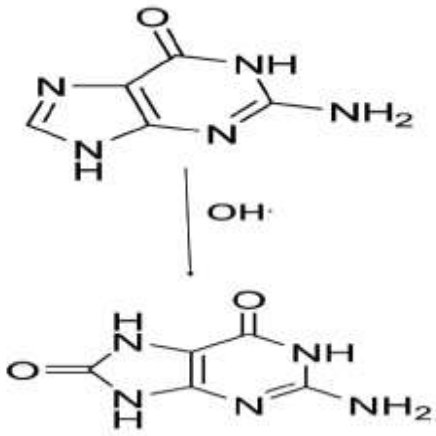

Figure 7. Oxidation of guanine to 8-oxoguanine (Baudin, 2020).

II. 5. Oxidative stress and pathologies

Like inflammation, oxidative stress is a phenomen implicated in several diseases: cancer, cataract, amyotrophic lateral sclerosis, acute pulmonary distress syndrome, pulmonary edema, and accelerated aging (**figure 8**). It is considered to be one of the factors potentiating the genesis of multifactorial diseases like diabetes and cardiovascular diseases (Favier, 2006; Liguori et *al.*, 2018).

According to Cederbaum (2017) the brain is the major target of ROS which play a big role in Alzheimer's disease, Parkinson's and other neurodegenerative diseases.

The relationships between oxidative stress and cancer appear to be very close; ROS cause DNA damage like 8-oxo-7,8-dihydro-2'-deoxyguanosine which is considered as a marker of oxidative stress (Jagoda and Dixon, 2020).

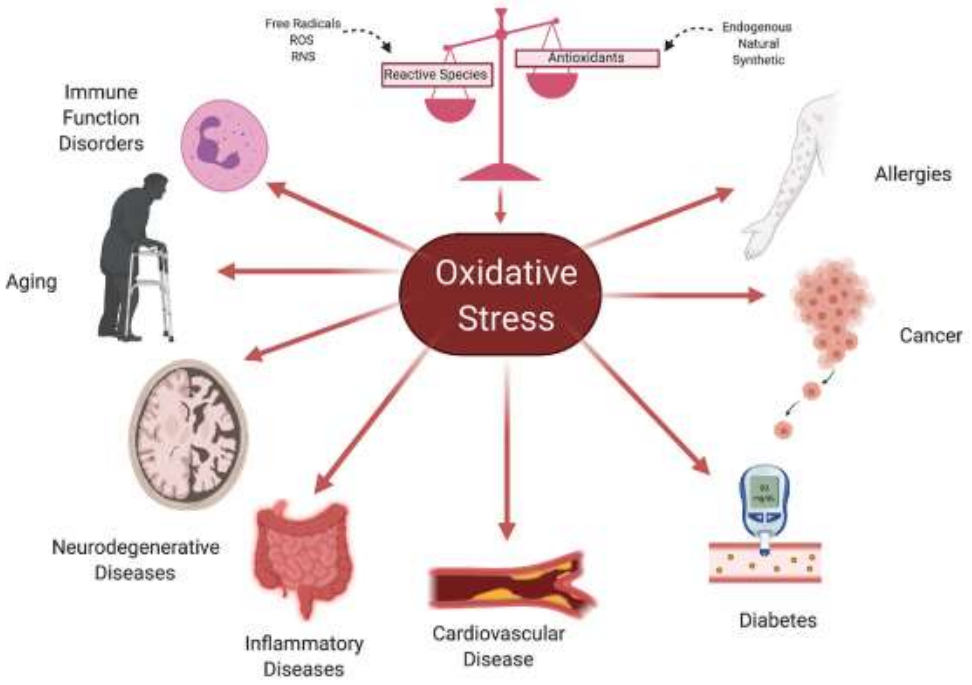

Figure 8. Oxidative stress related diseases (Fleming and Luo, 2021).

II. 6. Antioxidant mechanisms

In the case of oxidative stress, the cell must oppose the development and propagation of stress, by maintaining the balance between the production of ROS and their destruction by antioxidant defense systems. These antioxidant systems can be enzymatic or non-enzymatic antioxidants.

II. 6. 1. Enzymatic antioxidants

Superoxide dismutase

Superoxide dismutase (SOD) plays an important role in the dismutation of highly reactive $O_2^{\bullet -}$ into H_2O_2 and oxygen (**figure 9)** (Song and Zou, 2015). SOD exists in three distinct isoforms which are differentiated by their cellular localization and by their metal cofactor: a cytosolic form associated with copper ions and/or zinc CuZnSOD (SOD1), a mitochondrial form associated with manganese; Mn-SOD (SOD2) (Peng et *al.*, 2014).

Catalase

Catalase (CAT) is an enzyme responsible for converting H_2O_2 into water (H_2O) and oxygen (**figure 9**) using iron or manganese as a cofactor. Catalase is preferentially located in the peroxisome and in small quantities in the cytoplasm and mitochondria. The affinity of catalase for H_2O_2 is minor in case of low H_2O_2 concentration, but it becomes increasingly important when H_2O_2 contents are high (Gandhi and Abramov, 2012).

Glutathione peroxidase

Glutathione peroxidase (GPx) is an important intracellular enzyme that catalyzes the reduction of H_2O_2 using reduced glutathione (GSH) as a substrate (**figure 9**). It can also reduce other lipid peroxides to alcohols mainly in mitochondria and cytosol, its activity dependent on selenium (Francenia Santos-Sánchez et *al.*, 2019).

Glutathione reductase

Glutathione reductase (GR) an important enzyme in the reduction of glutathione disulfide (GSSG) to GSH (**figure 9**) using flavin adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (Espinosa-Diez et *al.*, 2015).

Glutathione S-transferase

Glutathione S-transferase (GST), enzyme that catalyzes the conjugation of glutathione with hydrophobic molecules and participates in the repair of damage to macromolecules caused by oxidative stress and the regeneration of oxidized sulfuric proteins (Z. Zhang et *al.*, 2017).

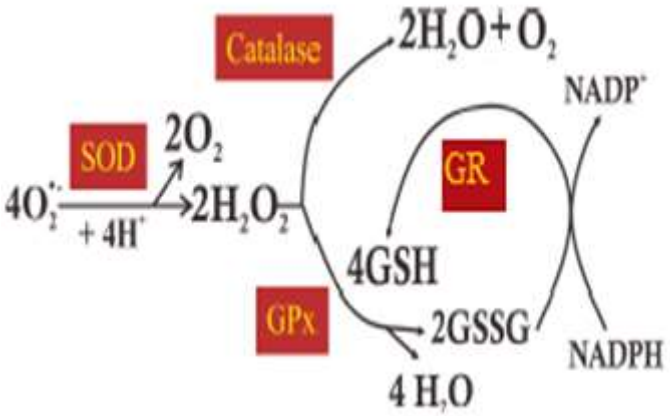

Figure 9. The major enzymatic antioxidants and their actions (Zulaikhah, 2017).

II. 6. 2. Non-enzymatic antioxidants

Vitamin

Vitamin E is a fat-soluble antioxidant that guards against ROS damage to cell membranes (Dasuri et *al.*, 2013). Vitamin E can intercalate in the lipid bilayer of membranes and reacts with the fatty acid radical and the pyroxyl radical thus ending the chain reaction of lipid peroxidation (Cederbaum, 2017).

Vitamin C or ascorbic acid is a water-soluble antioxidant and considered to be the most powerful natural antioxidant (Gülçin, 2012). It prevents the oxidation of low density lipoproteins, transfers hydrogen atoms to the peroxyl radical and inactivates $^1O_2$ (Francenia Santos-Sánchez et *al.*, 2019) and it also participates in the recycling of vitamin E (Gülçin, 2012; Song and Zou, 2015).

Vitamin B6 exerts an indirect antioxidant role by promoting the synthesis of cysteine from methionine and thus enhances the production of GSH (Dalto and Matte, 2017).

Glutathione

GSH is a tripeptide (glutamyl-cysteinyl-glycine) which plays an important role in cellular protection against oxidative stress. It exists in two forms: a very abundant reduced form (GSH), and an oxidized form (GSSG) (Nemmiche, 2016; Kamal et *al.*, 2019). The following two kinds of reactions include GSH:

• The thiol group of GSH serves as a major attack target for ROS (Song and Zou, 2015).

• GSH is a main source of hydrogen; it constitutes a substrate for GPx (Liguori et *al.*, 2018).

Trace element

Trace elements serve in particular as cofactors for antioxidant enzymes, for example selenium is the cofactor for GPx (Svoboda et *al.*, 2016); copper and zinc are cofactors of SOD (Bueno et *al.*, 2020).

Coenzyme Q10

A powerful fat-soluble antioxidant that enriches the mitochondrial oxidative chain and cell membranes. It has a ROS scavenger activity, thus allowing protection of membranes against lipoperoxidation (Dasuri et *al.*, 2013).

Polyphenols

Polyphenols group more than 8,000 molecules that have the same basic structure characterized by the presence of at least one 6-carbon aromatic ring that carries a variable number of hydroxyl functions (Ganesan and Xu, 2017; Elufioye et *al.*, 2019). These molecules which involve several classes (phenolic acids, flavonoids, anthocyanins, tannins and stilbenes) have shown great interest in human health while reducing the risk of certain pathologies such as inflammatory diseases, cardiovascular diseases, cancer , neurodegenerative diseases and microbial and viral infections (Bernstein et *al.*, 2018; Olivares-Vicente et *al.*, 2018).

As antioxidants, the primary function of polyphenols is to scavenge ROS and prevent or stop the spread of lipid peroxidation (Shahidi and Ambigaipalan, 2015). They can also act as a metal chelator thus preventing the Fenton reaction (Shahidi and Ambigaipalan, 2015; Jomova

et *al.*, 2017) and inhibitor of certain enzymes involved in ROS generation such as xanthine oxidase and cyclo-oxygenase (Jayasena et *al.*, 2013; Shahidi and Ambigaipalan, 2015).

II. 6. 3. Other antioxidants

There are other peroxidases like peroxiredoxins, which react with H_2O_2 and catalyze the reduction and detoxification of peroxynitrites and other organic hydroperoxides (Espinosa-Diez et *al.*, 2015), and thioredoxin reductase and thioredoxin which have a similar function of GSH and GPx (Cederbaum, 2017).

Bilirubin which results primarily from the catabolism of hemoglobin. It is able to scavenge peroxyl radicals and singlet oxygen, its cytoprotective activity is based on the inhibition of lipid and protein peroxidation (Grochot-Przeczek et *al.*, 2012).

Carotenoids represent groups of exogenous antioxidants that react with the products of lipid peroxidation blocking the chain of lipid peroxidation (El-Beltagi and Mohamed, 2013).

Proteins like albumin can trap directly on ROS or indirectly by uptake of transition metals by inhibiting the Fenton and Haber-Weiss reaction (Taverna et *al.*, 2013).

N-acetylcysteine, the derivative of the amino acid l-cysteine is considered a scavenger of free radicals (Aldini et *al.*, 2018) and it is also able to regenerate albumin (Altomare et *al.*, 2020).

III. *Hypericum tomentosum L.*

Hypericum tomentosum (*H. tomentosum*) was selected for its traditional use and its distribution in our country and for few studies in it.

III. 1. Description

There are over 450 species of trees, shrubs, and herbs in the genus *Hypericum*, which is a member of the Hypericeae family, and they may be found across the world's temperate zones (Mártonfiová et *al.*, 2014). The name *Hypericum* comes from *hypo* and *ereikn* or *erikn* meaning "plant that grows under heathers"; it could also come from *hyper* and *eikon* meaning "plant resembling a ghost's image or plant with an air of mystery" (Mederos-Molina, 2002).

H. tomentosum, commonly called "hchichat elhayra", is a perennial plant that is primarily found in the Mediterranean region. The word "*tomentosum*" refers to the plant's aerial section, which has paired white hairs (Henzelyová and Čellárová, 2018). This plant grows in meadows a little humid and at the edge of streams (**figure 10**).

The floristic characters of *H. tomentosum* can be defined as herbs with translucent glands containing hypericin, leaves are ovate, stemless and whorled, turn red when rubbed. This

reaction is due to hypericin, a red pigment. Flowers are bisexual, five sepals, and five yellow petals. The flowers bloom is from June until September (Asan, 2019).

III. 2. Botanical classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Phylum: spermatophytae
Subphylum: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Malpighiales
Family: Clusiaceae
Subfamily: Hypericoideae
Tribe: Hypericeae
Genus: *Hypericum*
Specie: *tomentosum* (Touafek, 2010).

III. 3. Phytochemistry and biological properties

Studies have shown that *Hypericum* species contains phloroglucinol, flavonoids, xanthone and terpenes (Touafek et *al.*, 2012a; Zubrická et *al.*, 2015).

Numerous significant chemical compounds with biological activity can be found in *H. tomentosum*. These compounds are naphthodianthrone (hypericin and pseudohypericin) which have an intense red color and phototoxic properties (Napoli et *al.*, 2018). Phloroglucinol derivatives are widely distributed in this genus and the major group of biologically active compounds in *H. tomentosum* comprises of flavonoids. Kaempferol, luteolin, myricetin, quercetin, hyperoside, quercitrin and isoquercitrin (Asan, 2021).

Hosni et *al.* (2008) reported that the major compounds of *H. tomentosum* essential oils are menthone (17.0%), n-octane (9.9%), caryophyllene (5.3%), pinene (5.2%), lauric acid (4.1%) and pinene (3.7%); Bouratoua et *al.*, (2016) identified 8 flavonoids and a phenolic acid from the butanol extract: dehydrokaempferol, luteolin, quercetin, apigenin, rutin quercetin-7-O-α-L-rhamnosyl-3-O-ß-D-glucoside , quercitrin, hyperoside and chlorogenic acid; and isolated 3 sterols stigmasterol, campesterol and sitosterol from the dichloromethane extract.

The genus *Hypericum* is used in traditional medicine thanks to its anti-inflammatory, antioxidant, antiviral and analgesic effects (Rouis et *al.*, 2012); it is used for the treatment of depression, ulcer, rheumatism and hysteria (Bouratoua et *al.*, 2016), burns, hemorrhoids, diarrhea, and ulcers (Asan, 2021).

EXPERIMENTAL

PART

MATERIALS AND METHODS

Materials and methods

I. Materials

I. 1. Reagents

All chemicals used in the present study were of analytical grade. 2,2'-diphenyle-1-picrylhydrazyl (DPPH•), β-carotene, tween 40, linoleic acid, dimethyl sulfoxide (DMSO), trichloroacetic acid (TCA),potassium ferricyanide[$K_3Fe(CN)_6$], ethylenediaminetetraacetic acid (EDTA), ferric chloride (III) ($FeCl_3$) , ferrous chloride **(**II) ($FeCl_2$), folin-ciocalteu reagent, vanillin, hydrochloric acid (HCl), gallic acid, quercetin, catechin, standard analytes, peroxidase, AAPH (2,2'-Azobis (2-amidinopropane) dihydrochloride), ABTS• (2,2'- azino-bis (3 -ethylbenzothiazoline -6- sulfonic acid)),ferrous sulfate ($FeSO_4$), ferrozine , Trolox, ascorbic acid, butylatedhydroxytoluene (BHT), Hanks' Balanced Salt Solution with $Ca^{2+}$and Mg^{2+} (HBSS2) and without $Ca^{2+}$and Mg^{2+} (HBSS1), heparin lithium, dextran, Histopaque®-1077, fMLP (formyl-Methionyl-Leucyl-Phenylalanine), cytochalasin B (CB), trypan bleu, elastase substrate: *N*-Methoxy-Suc-$(Ala)_2$-Pro-Val-*p*-Nitroanilide, 4- (2-hydroxyethyl) piperazineethanesulfonic acid (HEPES), myeloperoxidase substrate : 3, 3`, 5, 5`-tetramethylbenzidine (TMB), o-dianizidine, methylen blue, hexadecyltrimethylammonium bromide (HTAB), Triton X100, croton oil, carrageenan, aspirin ,indomethacin were purchased from Sigma-Aldrich (Darmstadt, Germany). Salts for buffer solution preparation were procured from Panreac (Panreac, Spain). Sodium carbonate (Na_2CO_3), sodium salicylate, aluminum chloride ($AlCl_3$) and organic solvents were purchased from Prolabo (Prolabo, France). Hydrogen peroxide and organic solvent were acquired from Riedel-de Haen (Illkirch, France). Ammonium formate, formic acid and acetone were obtained from Merck (Darmstadt, Germany).

I. 2. Animals

Female mice *Swiss albinos* provided by Algeria Pasteur Institute weighing between 25 and 30 g were used in the *in vivo* study. The animals were kept in polypropylene cages, with free access to water and food, at room temperature, under natural cycle light. The food is provided by the National Office of Livestock Foods in Bejaia.The food was taken away the day before the experiment, but free access to water was still permitted.

I. 3. Plant material

The plant *Hypericum tomentosum* (*H. tomentosum*) was harvested during flowering phase in June, 2020 from Serdj el ghoul region in Setif, in Algeria. It was identified by Pr. H. Laouer, Setif 1 University, Algeria. The aerial parts were dried at room temperature and in shadow. Then air-dried leaves were ground using an electric grinder in order to get a fine powder.

Figure 10. Photography of *Hypericum tomentosum* (self photo).

I. 4. Human blood

Before each experiment, fresh human blood from healthy adult donors who had not smoked or taken any medications for at least the previous fifteen days was drawn from their veins to isolate neutrophils.

I. 5. Microorganisms

The culture of *Candida albicans* (*C. albicans*), procured from university hospital center, Setif and *Escherichia Coli* (*E. Coli*), procured from laboratory of microbiology, Setif, were obtained after seeding of strains on sabouraud dextrose agar and nutrient broth medium, respectively. The culture was incubated at 37°C for 24 to 48 hours.

II. Methods

II. 1. Preparation of extracts

Methanolic extract (Met.E) of *H. tomentosum* was prepared as described by Bouriche et *al.* (2017) by maceration of powdered dried leaves at ambient temperature for 48 hours in

methanol–water solvent mixture (80:20, v/v) at a solid–liquid ratio of 1:10 (w/v) with continuous stirring. The hydro-methanolic extract was filtered and the residue was re-macerated in 50% methanol for the second time during 24hours. After filtration in the same condition, the filtrate was concentrated under reduced pressure at 40 °C in rotavapor (BÜCHI) and dried in oven to get red powder which was stored at -32 °C until use. The extraction's yield was calculated using formulation below:

$$Y\ (\%) = (M/\ M_0) \times 100$$

Y: yield of extraction

M: mass by gramof resulting extract

M_0: mass by gram of powder plant using for extraction

According to the protocol described by Gülçin et *al.* (2010), aqueous extract (Aq.E) of *H. tomentosum* was prapared. A weight of powdered dried leaves was boiling for 15 min at solid-liquid ration of 1:16. After cooling the mixture was filtrated two times successive the extract was lyophilized to obtain a red fine powered which was stored at -32 °C until use.

II. 2. Phytochemical analysis

II. 2. 1. Determination of total phenolic content

The total phenolics content of aqueous and methanolic extracts were determined as described by Li et *al.* (2007). Practically, 100 µL of extract with different concentrations were mixed with 500 µL of folin-ciocalteu's reagent 10%. After 4 min, 400 µL of Na_2CO_3 7.5% were added and the reaction mixture was incubated at ambient temperature and at obscurity for two hours, followed by the measuring of absorbance at 765 nm. Concentration on polyphenol of each extract was calculated from regression equation of gallic acid's standard calibration curve (**figure11**) and the results were expressed as microgram of Gallic Acid Equivalent per milligram of extract (µg GAE/ mg of extract). All samples were analyzed three times and the mean value was calculated.

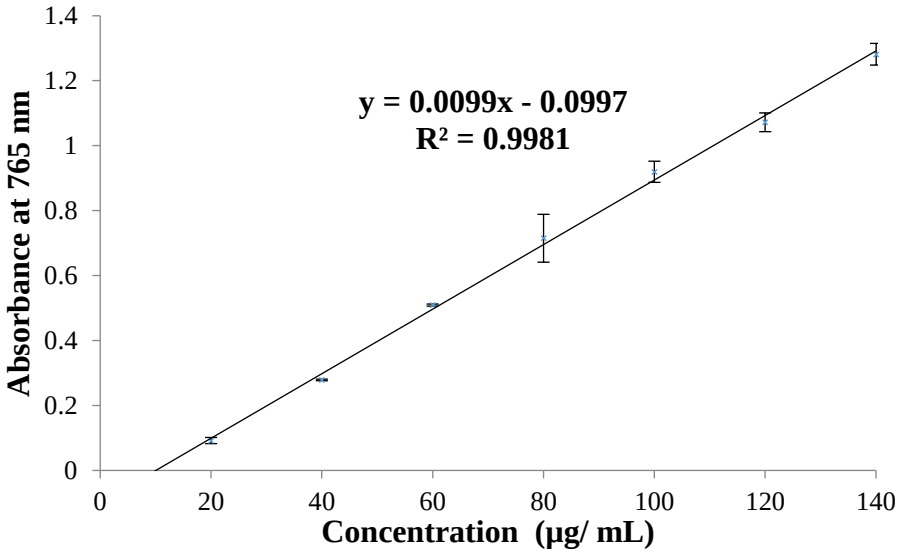

Figure 11. Standard curve of gallic acid, the values represent the means of 3 repetitions ± SD.

II. 2. 2. Determination of total flavonoids content

Total flavonoids were determined using colorimetric method according to Bahorun et *al.* (1996). A volume of 0.5 mL of different concentrations of sample was mixed with the same volume of $AlCl_3$ 2% prepared in methanol. After incubation for 10 min at ambient temperature and obscurity the absorbance was measured at 430 nm against blank containing the solvent instead of extract. The concentration of flavonoids was deduce from quercetin standard curve (**figure 12**) and the results were expressed as microgram of Quercetin Equivalent per milligram of extract (µg QE/ mg of extract).

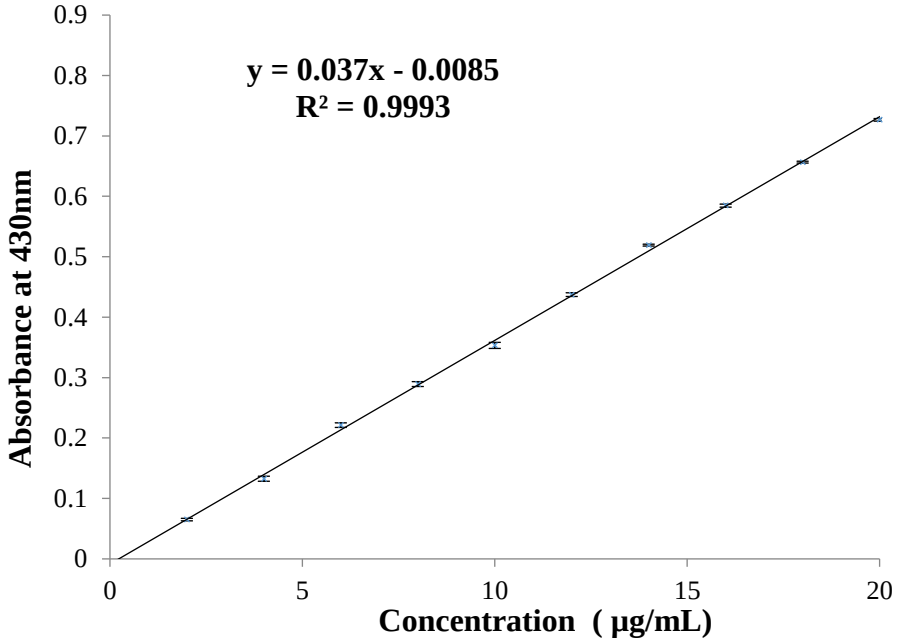

Figure 12. Standard curve of quercetin, the values represent the means of 3 repetitions ± SD.

II. 2. 3. Determination of total condensed tannins

The amount of condensed tannins of *H. tomentosum* Aq.E and Met.E was evaluated according to the method of Ghedadba et *al.* (2015). Briefly, 500 µL of extract were mixed with 750 µL of vanillin 4% dissolved in methanol. Then 375 µL of HCl 37% (12M) were added. The reaction mixture was incubated in water bath at 30°C for 20 min following by measurement of absorbance at 500 nm. The content of condensed tannin was calculated using equation of catechin'sstandard curve (**figure 13)** and the results were expressed as µg of Catechin Equivalent per mg of extract (µg CE/ mg of extract)

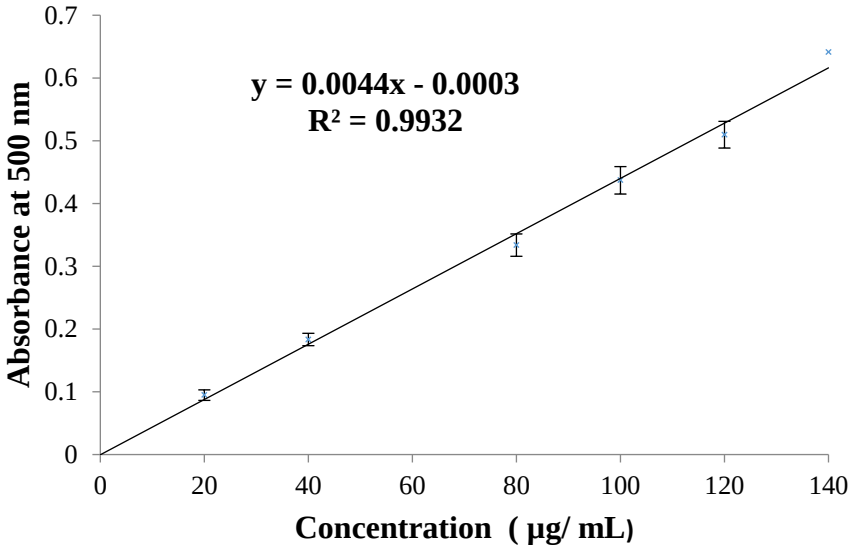

Figure 13. Standanrd curve of catechin, the values represent the means of 3 repetitions ± SD.

II. 2. 4. Mass spectrometer and chromatography conditions

53 of standards were quantitatively evaluated using a tandem mass spectrometer and an ultrahigh performance liquid chromatograph (UHPLC) of the Shimad zu-Nexera model. The autosampler (SIL-30AC model), column oven (CTO-10ASvp model), binary pumps (LC-30CE model), and degasser (DGU- 20A3R model) were all included in the reversed-phase UHPLC. To get the best separation for 53 phytochemicals and overcome the suppression effects, the chromatographic conditions were improved. On reversed phase Agilent Poroshell 120 EC-C18 model analytical column (150 mm 2.1 mm, 2.7 m), the chromatographic separation was carried out. The column temperature was fixed at 40°C. The elution gradient consisted of mobile eluent A (water + 5mM ammonium formate+0.1% formic acid) and eluent B (methanol + 5mM ammonium formate+0.1% formic acid). The following gradient elution profile was used: 20–100% B (0−25 min), 100% B (25−35 min), 20% B (35−45 min). The solvent flow rate was

maintained at 0.5 mL/min and injection volume was settled as 5 µL. The working conditions were determined as interface temperature; 350°C, DL temperature; 250°C, heat block temperature; 400°C, nebulizing gas flow (Nitrogen); 3 L/min and drying gas flow (Nitrogen); 15 L/min (Yilmaz, 2020).

II. 3. Antioxidant activity

II. 3. 1. DPPH•scavenging activity

As described by Bouriche et *al.* (2017), the DPPH• radical scavenging capacity of Aq.E and Met.E was assessed. Practically 0.5 mL of different concentrations of each extract were mixed with 0.5 mL of DPPH• 0.1 mM produced in methanol. The mixture was let to sit in the dark for 30 minutes. At 517 nm, the absorbance was measured. Trolox (0.5–60 µg/mL) was employed as the standard, and the following formula was used to compute the percentage of radical scavenging activity:

$$\text{DPPH}^{\bullet}\text{ scavenging activity (\%)} = (A_{control} - A_{sample}) \times 100/A_{control}$$

$A_{control}$**:** absorbance of the control reaction mixture without test compounds

A_{sample}**:** absorbance of sample reaction mixture with test compounds

II. 3. 2. Hydrogen peroxide scavenging activity

The capacity of Aq.E and Met.E to trap the non radicalar reactive specie H_2O_2 was performed as described by Müller (1985). Briefly, in 96 wells microplate, 20 µL of extracts in different concentrations were incubated with 100 µL of phosphate buffer saline (PBS) (pH = 5; 0.1 M) and H_2O_2 (20 µL; 10 mM) for 5 min at 37°C. After that, 30 µL of ABTS• (30 µL; 1.25 mM) and peroxidase (30 µL; 1 U/mL) were added. The mixture was incubated again for 15 min at 37°C. In this test, the C vitamin was used as the reference, and the absorbance was measured at 405 nm using a microplate reader.

II. 3. 3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of *H. tomentosum* Aq.E and Met.E was estimated by mixing 500 µL of $FeSO_4$ (1.5 mM), 350 µL H_2O_2 (6 mM) and 100 µL of extracts with different concentrations. After 5 min of incubation, 150 µL of sodium salicylate (20 mM) were added and the reaction medium was incubated again for 1 hour at 37 °C following by reading the absorbance at 562nm length wave. The vitamin C (20 – 500 µg/mL) was used as standard (Sharma et *al.*,2013).

II. 3. 4. Reducing power

The reducing power of both extracts was determined by incubation of 200 µL extracts at different concentrations with 425 µL of PBS (0.2 M, pH= 6.6) and 625 µL of [$K_3Fe(CN)_6$] 1% (prepared in PBS) for 20 min at 50°C. After cooling, 625µL of TCA 10% in PBS was added. 625 µL of each mixture was transferred in other tubes which contain 625 µL of distilled water and 125 µL $FeCl_3$ 0.1%. The absorbance was measured subsequently at 700 nm against blank without extracts. BHT (2.5 – 50 µg/mL) was used as standard antioxidant (Bougatef et *al.*, 2009).

II. 3. 5. Ferrous ion chelating activity

Ferrous ion chelating activity was evaluated as described by Bouriche et *al.* (2017). Briefly, 50 µL of $FeCl_2$ (0.6 mM) were added to 700 µL of both extracts at various concentrations. After 5 minutes of incubation, the reaction was started by adding 50 µL of ferrozine (5 mM), produced in 80% methanol. The reaction required another 10 minutes of incubation in the dark in order to create the complex (Fe (II)-Ferrozine), which is violet in color and has a high absorbance at 562 nm. The standard chelator utilized was EDTA (1-30 µg/mL). The following equation was used to determine the chelating activity of aqueous, methanolic extracts:

Chelating activity (%) = $(A_{control} - A_{sample}) \times 100/A_{control}$

$A_{control}$**:** absorbance of the control

A_{sample}**:** absorbance of sample

II. 3. 6. Anti-hemolytic activity

According to the method described by Girard et *al.* (2006), the anti-hemolytic action of *H. tomentosum* extracts was evaluated. Summarily, blood from female mice was collected through a direct heart puncture using a heparinized syringe, and it was diluted to 2% in physiological buffer (NaCl 125 mM, sodium phosphate 10 mM, pH 7.4). 120 µL of mice erythrocyte solution (2%) was pre-incubated in a 96-well plate for 30 min at 37 °C with 60 µL of various doses of Aq.E and Met.E (5, 10, 25, and 50 µg/ mL) or Trolox (25 and 50 µg/ mL). After adding to each well 120 µL of AAPH (120 mM) prepared in cold physiological buffer, the mixture was incubated at 37°C. For 4 hours, the kinetics of hemolysis were observed by measuring turbidity decreases at 630 nm.

II. 3. 7. β-Carotene bleaching method

The capacity of extracts to prevent discoloration of β-carotene was audited by method described by Bougatef et *al.* (2009). 0.5 mg of β-carotene were dissolved in 1mL of chloroform, then 25 µL linoleic acid and 200 mg of tween-40 were added. The chloroform was evaporated under vacuum at 45°C following by addition of 100 mL of oxygenated distilled water and the

mixture was vigorously stirred. 2.5 mL of this mixture was transferred in tubes which contain 500 µL of aqueous and methanolic extract (2 mg/ mL) or standard BHT (2 mg/mL). The absorbance was measured immediately (t=0 min) at 470 nm. Subsequently, the reaction mixtures were incubated at 50°C. The absorbance was measured every 15 min for 2 hours (until t=120 min) against a blank that contain linoleic acid, tween-40, oxygenated distilled water and sample. All samples were assayed in triplicate. The β-carotene bleaching inhibition was calculated using the following formulation:

$$\text{Inhibition (\%)} = \left[1 - \frac{A_0 - A_{120}}{A_{C0} - A_{C120}} \right] \times 100$$

A_0: absorbance of sample at t= 0min. A_{120}: absorbance of sample at t= 120min.

A_{C0}: absorbance of controle at t= 0min. A_{C120}: absorbance of controle at t= 120min.

II. 4. Anti-inflammatory activity

II. 4. 1. *In vitro* anti-inflammatory activity

II. 4. 1. 1. Human neutrophil isolation

The PMNs were freshly isolated before each experience from healthy volunteers not smokers and didn't take any remedy according to method described by Bouriche et *al.* (2016). Ten milliliters of blood levied in tube of centrifugation which contain lithium heparinate (5 U/ mL) as anticoagulant. First, the red blood cells were separated from other cells by sedimentation in presence of 2 mL of dextran 10%. After an hour of sedimentation at ambient temperature, the plasma which enriched by leukocyte was recuperated and transferred in centrifugation tube of polypropylene following by injection of 2mL of Histopaque®. Then tube was centrifuged at 400 g during 25 min at 4°C. The remaining red blood cells were eliminated by a serial hypotonic lysis by addition of 1 mL of fresh cold distilled water for 30 sec. Subsequently, 5 mL of fresh HBSS1 were added. The cellular suspension was centrifuged for 10 min at 400 g at 4°C. The serial hypotonic lysis was repeated until total elimination of erythrocytes. In the end of the last centrifugation, neutrophils were suspended in HBSS1 and conserved in ice bath (**figure 14**).

II. 4. 1. 2. Cell viability

Prior the investigation of the effects of *H. tomentosum* extracts on neutrophils fonction, the cytotoxic effect on these cells was tested using trypan blue exclusion test. This test was performed by incubation of 200 µL of the isolated cells ($3x10^6$ cells/ mL) in the presence of 50 µL of different concentrations of aqueous and methanolic extract (25, 50, 100, 200 µg/mL) for

30 min at 37°C, then 250 µL trypan blue (0.4%) were added and incubated again for 10 min. the counting was realized using microscope under X40 (Kernouf et *al.* 2018). The percentage of cellular viability was determinate according to following equation:

Viability% = (TC-CC) x100/ TC

TC: total cells (viable and not viable cells)

CC: colored cells (not viable cells**)**

II. 4. 1. 3. Determination of elastase activity

To investigate the extracts' ability to modulate the immune system, an experiment of neutrophil elastase activity was performed. According to Bouriche et *al.* (2016), the elastase activity was assessed in the supernatant secreted from the cells. Elastase was created by incubating a suspension of PMNs (5.5 x 10^6 cells/mL) for 15 minutes at 37°C with fMLP (10^{-6} M) and CB (10^{-5} M) as stimuli. 75 µL of the elastase-rich supernatant were distributed in a microplate well after 400 g of centrifugation at 4°C for 15 minutes. Then, 50 µL of various concentrations of aqueous and methanolic extract (0.5-50 µg/mL) or HBSS1 (Control) were added, and the mixture was incubated for 10 min at 37°C. Thereafter, 75 µLof the synthetic elastase substrate N-MeO-Suc- $(Ala)_2$-Pro-Val-p-nitroanilide (5 x 10^{-4} M) were added. This substrate was previously prepared in methyl 2-pyrulidone at a concentration of 5 x 10^{-2} M and then diluted in a HEPES medium (0.1 M; pH 7.4) to get a concentration (5 x 10^{-4} M). The proportion of elastase activity compared to the control, which was assumed to have 100% of the activity, was used to express the results.

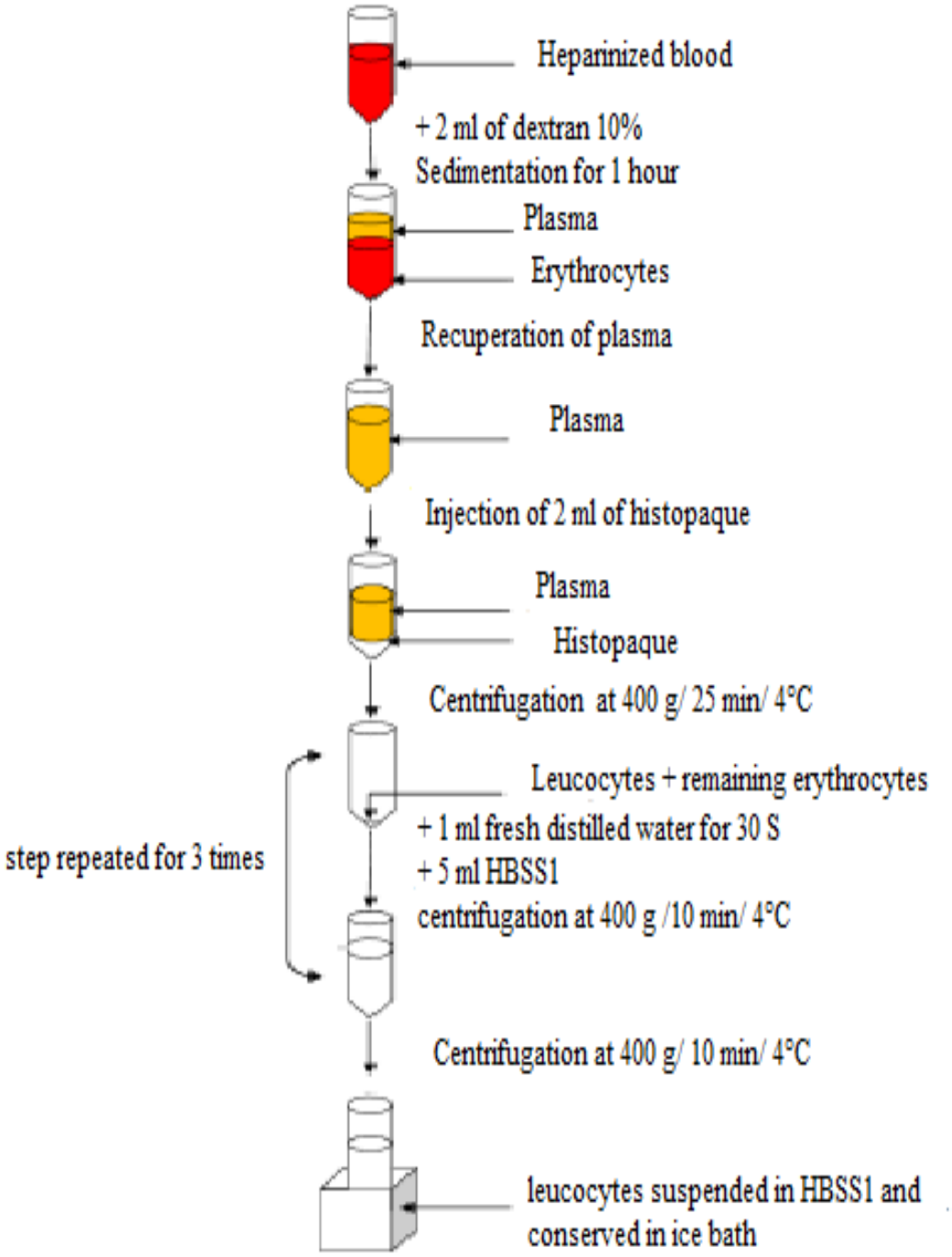

Figure 14. Steps of neutrophils isolation from human blood.

II. 4. 1. 4. Determination of myeloperoxidase activity

II. 4. 1. 4. 1. Determination of myeloperoxidase activity using tetramethylbenzidine as substrate

The effect of extracts on myeloperoxidase (MPO) activity was evaluated according to method of Wanikiat et *al.* (2008) which focus on oxidation of 3, 3`, 5, 5`-tetramethylbenzidine (TMB) by MPO in presence of H_2O_2. The production of this enzyme was induced by incubation of neutrophils ($5,5x10^6$ cells/mL) in HBSS2 at 37°C for 15 min in presence of fMLP/cytochalasin B (10^{-6} M/10^{-5} M) following by centrifugation at 400 g at 4°C for 15 min. In microplate well, 20 µL of supernatant were incubated with 30 µL of extracts or HBSS2 for 10 min at 37°C.Then, 25 µL of substrate (1.6 mM in DMSO) and 100 µL of H_2O_2 (0.003% prepared in PBS (50 mM, pH 5,4, 0,05% hexadecyltrimethylammonium bromide). The microplate was incubated again for 5 min and the reaction was stopped by addition of 50 µL of sulfuric acid (2 N). The absorbance was determined using microplate reader at 450 nm. Enzymatic activity was expressed on percentage compared with control which represent 100% activity.

II. 4. 1. 4. 2. Determination of myeloperoxidase activity using o-dianizidine as substrate

MPO was released by incubation of neutrophils ($4x10^6$ cells/mL) in HBSS2 at 37°C for 15 min in presence of fMLP/Cytochalasin B (10^{-6} M/10^{-5} M) following by centrifugation at 400 g at 4°C for 15 min. The effect of both extracts on MPO activity was evaluated by incubation of 25 µL of supernatant enriched by this enzyme in the presence of different concentrations of Aq.E and Met.E (25 µL) for 15 min followed by addition of 25 µL o-dianizidine (4 mg/mL) and 25 µL of H_2O_2 (0.003% prepared in HBSS2). The microplate was incubated for 15 min at 37°C, the absorbance was measured at 450 nm using microplate reader (Boussif et *al.*, 2016).

II. 4. 1. 5. Phagocytosis test

500 µL of PMNs (10^6 cells/ mL) were incubated with 50 µL of both extracts at different concentrations (5, 10, 25, µg/mL) at 37°C for 10 min. Then, 500 µL of *C. albicans* (10^6 cells/ mL) to obtained a ration 1:1 (yeast/ neutrophils), and 50 µL of serum, to serve as opsonize, were added. After incubation under agitation at 37°C during 50 min, 2 mL of methylene blue (0.001%) were added and the mixture were incubated again for 10 min following by centrifugation at 400 g for 10 min. The two third (2/3) of supernatant were eliminated and the remains of supernatant was homogenized and 20 µL of this homogenate were deposited on

Thoma cell to count the number of yeasts ingested by neutrophils under X40 of optic microscope. The results were expressed as percentage of phagocytosis where the control represent 100% phagocytosis activity (Ortega et *al.*, 1993).

II. 4. 1. 6. Anti-bactericidal activity

The ability of extract to inhibit the bactericidal activity of neutrophils was tested with specie of *Escherichia Coli* and for that PMNs ($5x10^5$ cells/mL) were incubated with extracts, at different concentrations (5, 10 and 25 µg/mL) or HBSS1 for control at 37°C. After 15 min of incubation, the mixture was centrifuged and the supernatant was eliminated and the cells were washed by 1 mL of PBS (50 mM, 125 mM NaCl, pH= 7.4). Then the neutrophils were incubated again with living and opsonized *E. coli* ($5x\ 10^4$ cells/ mL) and were stimulated with fMLP (1 µM) for 25 min under agitation at 37°C. After 25 min, the mixture was conserved directlyin the ice bath and the lysis of neutrophils was affected by Triton X100 for 10 min at ambient temperature to liberate phagocytic and not lysed bacteria. To count the survival *E.coli*, 15 µL of mixture were plated on nutritive agar and conserved overnight at 37°C to get colonies (Boussif et *al.*, 2016). The results were expressed as number of colonies.

II. 4. 2. *In vivo* anti-inflammatory activity

II. 4. 2. 1. Toxicity test

Before any investigation of plant's effect on mice, a toxicity test must be done to determine the no toxic dose according to organization for economic co-operation and development (OECD) guidelines 420 (OECD, 2001), for that the mice were subdivided after fasting overnight, on 3 groups: group 1 received orally distilled water, group 2 and 3: received orally 2 g/kg of aqueous and methanolic extracts dissolved in distilled water, respectively. The mice were monitored for mortality and general behavior (agitation, diarrhea, asthenia, appetite and convulsion) for a period of 14 days. In the case of survival of mice, same test was repeated but with dose of 5 g/Kg of aqueous and methanolic extracts.

II. 4. 2. 2. Croton oil induced ear edema assay

To evaluate the *in vivo* anti-inflammatory activity of *H. tomentosum*, a cutaneous inflammation in the ear was induced by application of 15 µL of acetone- water (1:1) containing 80 µg of croton oil in the inner surface of each mouse's right ear. The extracts 2 mg/ear and indomethacin 0.5 mg/ear were dissolved in the mixture. The thickness of mice's ear was measured by dial caliper before and after 6 hours of application. The edema was expressed as increased ear thickness due to croton oil treatment (Manga et *al.*, 2004). The percentage of

inhibition of edema was defined compared with control group (which received only croton oil in the mixture acetone-water) using the formulation below:

Inhibition (%) = (Δ control – Δ treated) x100/Δ control

Δ control : the difference of control group's ear thickness after and before edema

Δ treated : the difference of treated group's ear thickness after and before edema

II. 4. 2. 3. Carrageenan-induced paw edema assay

Carrageenan-induced edema assay was conducted on overnight fasting mice according to Mohammedi et *al.* (2018) and Amroun et *al.* (2021). Mice were divided into seven groups of five animals each as followed:

Group 1 and group 2: considered as a negative and positive control, respectively and received distilled water, group 3: was considered as standard group and received indomethacin 20 mg/kg, group 4, 5, 6 and 7 represented the testing groups and received the Aq.E and Met.E at doses of 200 mg/Kg and 400 mg/kg for each extract, respectively. 30 min after the oral administration of the substances or distilled water, the edema was induced into the sub-plantar tissue of the right paw by injection of 0.025 mL of carrageenan suspension (1% of carrageenan in normal saline) in all groups excluding negative control which was injected by normal saline. The paw thickness was measured using a dial caliper, before the induction of the edema, and every hour during the four following hours. After this time, all the treated animals were sacrificed and the hind paws were amputated and weighed.

II. 4. 2. 4. Air pouch assay

The air pouch was induced by the subcutaneous injection of 3 mL of sterile air on the dorsum. The pouches were re-inflated by injection of 1,5 mL sterile air on day 3 and day 6. On 7^{th} day, the mice received in the pouches 1 mg/mL of Aq.E and Met.E or 0.1 mg/mL of indomethacin. However, the control group was received 1 mL of sterile saline and then 0.1 mL of carrageenan (1%) were onjected into the pouch after 1 hour of treatment with test agents. The mice were sacrificed by cervical dislocation after 4 hours of treatment and 1 mL of saline was injected in the pouches which were opened after light message to collect the exudates. Leukocytes were counted using optic microscope under objective X 40 after dilution (1:10) with Türk's solution (Kernouf et *al.*, 2018). The percentage of inhibition of migration's neutrophils was calculated as following formula:

Inhibition (%) = (number of PMNs in positive control – number of neutrophils of tested group) x 100/ number of PMNs in positive control.

II. 4. 2. 5. Analgesic test

The analgesic effect vis a vis pain caused by acetic acid on mice of both extracts was evaluated as described by Epa et *al.* (2018). Practically, the mice were subdivided into six groups after fasting overnight.

Group 1 (control): orally received distilled water.

Group 2: orally received 100 mg/kg of aspirin

Group 3 and 4: orally received 200 mg/kg and 400 mg/kg of Aq.E respectively.

Group 5 and 6: orally received 200 mg/kg and 400 mg/kg of Met.E, respectively.

After 1h of treatment, the mice were injected intraperitoneal by acetic acid 0.6% prepared in sterile saline and the number of abdominal cramp or contortion was counted for 25 min. The percentage of inhibition of pain was calculated as following formula:

Inhibition (%) = (Ac-At) x 100/Ac

Ac: average of contortion's number in control group

At: average of contortion's number in treated group

III. Statistical analysis

Results obtained are expressed as means ± SD (standard deviation) where experiments were performed in triplicate. Or as means ± SEM (standard error means). The analysis of variance was performed by one-way ANOVA analysis and then the Tukey test for multiple comparisons was performed using GraphPad Prism 8 software. The difference was considered significant for $p<0.05$. The IC_{50} (Concentration corresponding to 50 % of inhibition), EC_{50} (The concentration corresponding to 50 % of effectiveness) and HT_{50} (half hemolysis time) were calculated from plot of linear regression.

RESULTS AND DISCUSSION

Results and discussion

I. Extraction and phytochemical analysis

I. 1. Yield of extraction

Extraction is a very important step for molecules' isolation and identification. The use of leave's powders allows to make the sample more homogeneous, increase the surface of contact with solvent and render its penetration inside vegetal cells easy (Seidel, 2012; Jovanović et *al.*, 2017).

In this study, two methods of extractions were used: decoction and maceration. The decoction allows to extract preferentially polar compounds and some ostensibly hydrophobic compounds because the polarity of water decreases at high temperature (Jones and Kinghorn, 2005). Additionally, water at high temperature induce cells perturbation by facilitate solvent's penetration and molecule's solubilization (Albano and Miguel, 2011). However, the heat brings about degradation of thermos-labile molecules, which explain the progress of decoction in short duration (Ng et *al.*, 2020).

The maceration is a very simple extraction method, which could be used for extraction of thermo-labile components (Zhang et *al.*, 2018). To extract as many chemicals as possible, an aqueous alcoholic mixture was used. This is based on the fact that alcohol-based solvents can make cell walls more permeable, allowing for the effective extraction of significant amounts of polar and medium- to low-polarity contents (Seidel, 2006). The maceration was affected under agitation in the goal of accelerate the process of extraction and minimize the time of contact between solvent and extract. In addition, the progress of maceration at ambient temperature and the elimination of methanol under vacuum allows to obtain maximum of molecules and prevent their degradation or denaturation (Ng et *al.*, 2020). The goals of second maceration are extract the maximum molecules and assure an exhaustive extraction.

The maceration of *H. tomentosum* in aqueous-methanolic solvent give a paste extract with red dark color and high yield 27.34%. However, the decoction in distilled water gives a powder extract with light red color and yield of 21.06% (**table 2**). According to study of Briskin and Gawienowski (2001) and study of Zobayed et *al.* (2006) the red color of the extracts caused by richness of this plant by hypercin groups which present primarily on the margins of leaves and flower petals of this genus. The difference in yields between hydro-methanolic and aqueous

extract may contribute to many factors such as the type of solvent, temperature, time and pH (Azahar et *al.*, 2017).

Table 2. Aspects, colors and yields of extracts.

Extract	Aspect	Color	Yield
Hydro- methanolic	paste	dark red	27.34%
Aqueous	powder	light red	21.06%

I. 2. Phytochemical analysis

I. 2. 1. Total polyphenols, flavonoids and condensed tannins

In this study, the dosage of polyphenols, flavonoids and condensed tannins was performed, because the majority of pharmacological effects of medicinal plants are attributed for these compounds (Ghedadba et *al.*, 2015; Jomova et *al.*, 2017; Farahmandfar et *al.*, 2019).

The dosage of polyphenols using folin-ciocalteu reagent's shows that aqueous and methanolic extracts contain 303.10 ± 5,14 and 409.67 ± 15.06 µg GAE/ mg of dried extract, respectively. Another study carried-out by Makarova et *al.* (2021) shows that content on polyphenol of *Hypericum perforatum* flower's ethanolic extract was 371 mg GAE/g of extract. This result is close to the obtained results. Flavonoids, major class of polyphenol, were determined by aluminum chloride. The result shows that total flavonoid of aqueous and methanolic extract arrive until 53.56 ± 1.74 and 74.02 ± 0.57 µg QE/mg dried extract, respectively. Thus, these results show the richness of both methanolic and aqueous extract on polyphenols and flavonoids compuonds. However, *H. tomentosum* methanolic extract is richer than aqueous extract, these results can be explained by ability of methanol to increase the membrane permeability and extract polar, medium polar and low-polarity molecules (Seidel, 2006).

In vanillin assay, condensation of flavonols with vanillin in acidic medium leads to the formation of colored carbonium ions (Dai and Mumper, 2010). The monomeric flavanol catechin is frequently used as a standard. The amount of condensed tannins, revealed by vanillin, is 117.29 ± 5.06 and 163.7 ± 8.97 µg CE/mg of extract in aqueous and methanolic extract, respectively (**table 3**), the Aq.E has a low amount of condensed tannin compared with

Met.E. Indeed, the condensed tannins with high molecular weight are insoluble in aqueous phase (Cheynier, 2005).

Table 3. Total polyphenols, flavonoids and condensed tannins present in Aq.E and Met.E of leaves of *H.tomentosum*.

	Polyphenols (µgGAE/mg)	**Flavonoids (µgQE/mg)**	**Condensed tannins (µgCE/mg)**
Aq.E	303.10 ± 5.14	53.56 ± 1.74	117.29 ± 5.06
Met.E	409.67 ± 15.06	74.02 ± 0.57	163.7 ± 8.97

I. 2. 2. The LC–MS/MS analysis of *H.tomentosum* extracts

The phenolic identification and quantification of *H. tomentosum* Aq.E and Met.E were determined using liquid chromatography coupled with mass spectrometry (LC- MS/MS). This method allowed to identify ten acids, fourteen flavonoids, one biflavonoid and two phenyl aldehydes. Retention periods and m/z values were compared to mass spectra of commercial standards evaluated under similar circumstances **(table 4**).

The quinic acid was the most abundant acid present in both extracts its amounts in aqueous extract were richer than methanolic extract. Furthermore, chlorogenic acid was the second most prevalent acid compound after quinic acid with almost same concentration in Aq.E and Met.E.

Flavonoids are the most abundant polyphenols in diet, they can be divided into flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (Olivares-Vicente et *al.*, 2018).They are one of the main chemical classes already identified in *Hypericum s*pecies (Ramalhete et *al.*, 2016; Asan, 2021). Quercitrin presents with great amount in Aq.E and Met.E (281.86 mg/g of extract, 316.822 mg/g of extract, respectively). This high amount of quercitrin in both extracts makes them a natural source of this glycoside flavonoid. Flavan3-ol groups were identified in this study, the results show that the extracts are rich by epicatechin, its amount arrive until 169.28 mg/g of Aq.E and 154.99 mg/g of Met.E.

Table 4. Phenolic compounds in Aq.E and Met.E determined by LC-MS-MS.

No	Analytes	RT[a]	M.I. (m/z)[b]	F.I. (m/z)[c]	Quantitative Results (mg analyte/g extract)	
					Aq.E	Met.E
1	Quinic acid	3.0	190.8	93.0	64.13	47.736
2	Fumaric aid	3.9	115.2	40.9	2.305	1.183
3	Aconitic acid	4.0	172.8	129.0	N.D.	N.D.
4	Gallic acid	4.4	168.8	79.0	0.103	0.074
5	Epigallocatechin	6.7	304.8	219.0	N.D.	N.D.
6	Protocatechuic acid	6.8	152.8	108.0	0.931	0.847
7	Catechin	7.4	288.8	203.1	0.945	0.322
8	Gentisic acid	8.3	152.8	109.0	N.D.	N.D.
9	Chlorogenic acid	8.4	353.0	85.0	41.244	42.068
10	Protocatechuic aldehyde	8.5	137.2	92.0	0.04	0.018
11	Tannic acid	9.2	182.8	78.0	N.D.	N.D.
12	Epigallocatechin gallate	9.4	457.0	305.1	N.D.	N.D.
13	4-OH Benzoic acid	10.5	137,2	65.0	N.D.	N.D.
14	Epicatechin	11.6	289.0	203.0	169.28	154.99
15	Vanilic acid	11.8	166.8	108.0	N.D.	N.D.
16	Caffeic acid	12.1	179.0	134.0	0.135	0.082
17	Syringic acid	12.6	196.8	166.9	N.D.	N.D.
18	Vanillin	13.9	153.1	125.0	0.029	N.D.
19	Syringic aldehyde	14.6	181.0	151.1	N.D.	N.D.
20	Daidzin	15.2	417.1	199.0	N.D.	N.D.
21	Epicatechin gallate	15.5	441.0	289.0	N.D.	N.D.
22	Piceid	17.2	391.0	135/106.9	N.D.	N.D.
23	*p*-Coumaric acid	17.8	163.0	93.0	0.044	0.052
24	Ferulic acid-D3-IS[h]	18.8	196.2	152.1	N.A.	N.A.
25	Ferulic acid	18.8	192.8	149.0	N.D.	N.D.
27	Sinapic acid	18.9	222.8	193.0	0.393	0.619
28	Coumarin	20.9	146.9	103.1	N.D.	N.D.

[a]R.T: retention time, [b]MI (*m/z):* molecular ions of the standard analytes (m/z ratio), [c]FI (*m/z):* fragment ions, N.D.: not detected, N.A: not applicable.

Table 4. Phenolic compounds in Aq.E and Met.E determined by LC-MS-MS. (Continued)

No	Analytes	RT[a]	M.I. (m/z)[b]	F.I. (m/z)[c]	Quantitative Results (mg analyte/g extract)	
					Aq.E	Met.E
29	Salicylic acid	21.8	137.2	65.0	0.016	0.011
30	Cynaroside	23.7	447.0	284.0	1.251	1.37
31	Miquelianin	24.1	477.0	150.9	N.D.	N.D.
32	Rutin-D3-IS[h]	25.5	612.2	304.1	N.A.	N.A.
33	Rutin	25.6	608.9	301.0	N.D.	N.D.
34	isoquercitrin	25.6	463.0	271.0	7.098	10.326
35	Hesperidin	25.8	611.2	449.0	N.D.	N.D.
36	*o*-Coumaric acid	26.1	162.8	93.0	N.D.	N.D.
37	Genistin	26.3	431.0	239.0	N.D.	N.D.
38	Rosmarinic acid	26.6	359.0	197.0	0.125	0.155
39	Ellagic acid	27.6	301.0	284.0	N.D.	N.D.
40	Cosmosiin	28.2	431.0	269.0	N.D.	N.D.
41	Quercitrin	29.8	447.0	301.0	281.86	316.822
42	Astragalin	30.4	447.0	255.0	0.536	0.873
43	Nicotiflorin	30.6	592.9	255.0/284.0	N.D.	N.D.
44	Fisetin	30.6	285.0	163.0	N.D.	N.D.
45	Daidzein	34.0	253.0	223.0	N.D.	N.D.
46	Quercetin-D3-IS[h]	35.6	304.0	275.9	N.A.	N.A.
47	Quercetin	35.7	301.0	272.9	2.207	1.017
48	Naringenin	35.9	270.9	119.0	0.026	0.017
49	Hesperetin	36.7	301.0	136.0/286.0	0.153	0.064
50	Luteolin	36.7	284.8	151.0/175.0	0.125	0.16
51	Genistein	36.9	269.0	135.0	N.D.	N.D.
52	Kaempferol	37.9	285.0	239.0	0.091	0.034
53	Apigenin	38.2	268.8	151.0/149.0	0.032	0.021
54	Amentoflavone	39.7	537.0	417.0	0.007	0.038
55	Chrysin	40.5	252.8	145.0/119.0	N.D.	0.002
56	Acacetin	40.7	283.0	239.0	0.011	0.061

[a]R.T: retention time, [b]MI (*m/z):* molecular ions of the standard analytes (m/z ratio), [c]FI (*m/z):* fragment ions, N.D.: not detected, N.A: not applicable.

Amentoflavone, a biflavonoid, was also detected. When it comes to phenol aldehydes, protocatechuic aldehyde was presented with lower amount while traces of vanillin were found only in Aq.E but not in Met.E (**figure 15**).

When comparing the molecules identified in this plant in the current study with literature it was found that luteolin, quercetin, quercitrin and chlorogenic acid were previously identified in *H.tomentosum* (Bouratoua et *al.*, 2016) while quinic acid, fumaric acid, protocatechuic acid, gallic acid, caffeic acid, *p*-coumaric acid, sinapic acid, salicylic acid, rosmarinic acid, epicatechin, catechin, isoquercitrin, naringenin, hesperitin, cyranoside, astragalin, kaempferol, apigenin, acacetin, chrysin, amentoflavone, protocatechuic aldehyde and vanillin were identified and quantified for the first time in plant extracts.

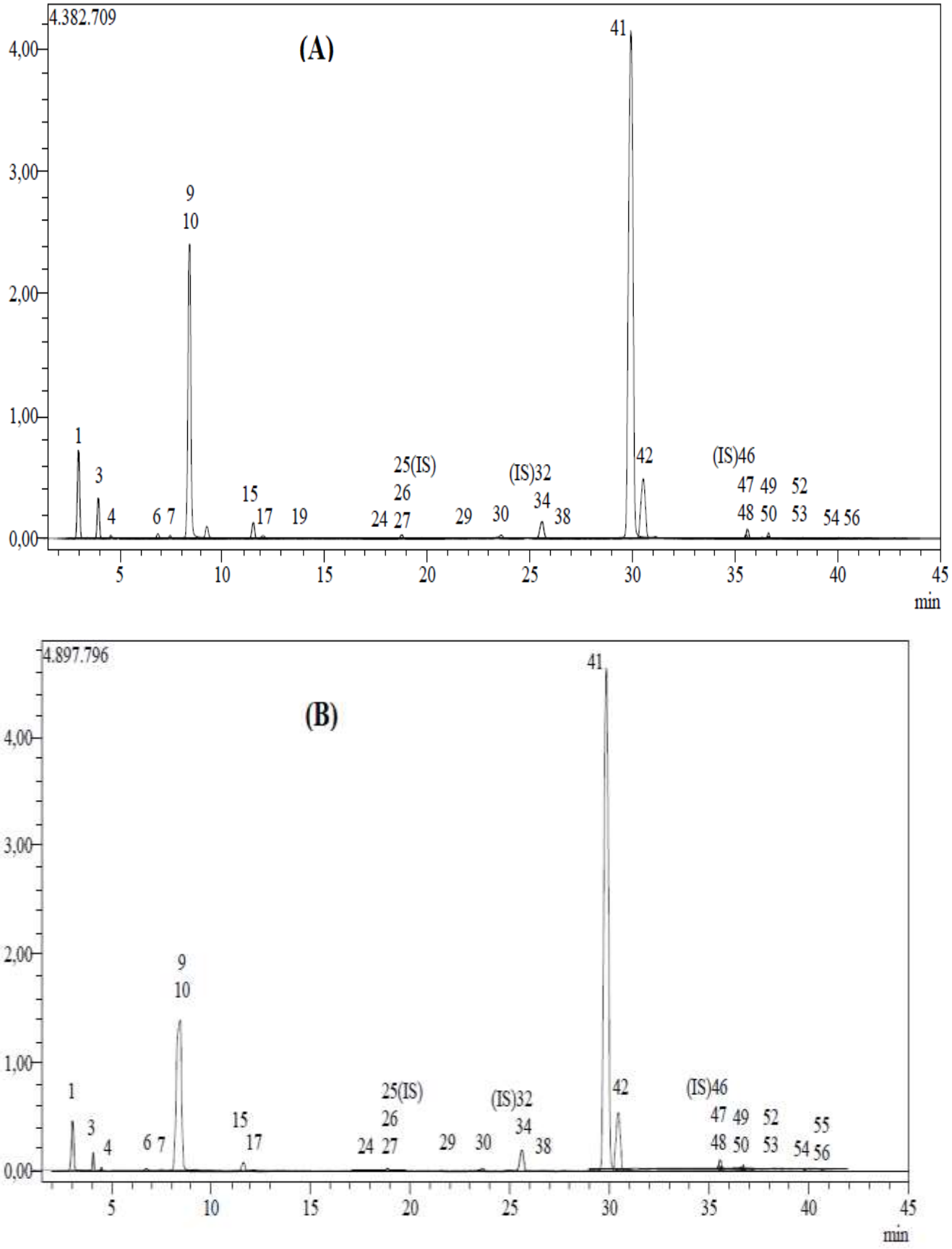

Figure 15. LC-MS-MS chromatograms of phenolic compounds in *H. tomentosum* aqueous extract (A) and methanolic extract (B).

II. Antioxidant activity

II. 1. DPPH• scavenging activity

DPPH• assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH•. This technique is frequently used to assess the capacity of sample to scavenge free radicals. It is a stable nitrogen-centered free radical, and when reduced by hydrogen or an electron donation, its color changes from violet to yellow. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Fathi and Ebrahimzadeh, 2013). In this study, both Aq.E and Met.E exerted a potent DPPH• scavenging (**figure 16**). Only 15 µg/mL of Met.E can scavenge up to 90% of DPPH• radical with IC_{50} = 5.85 ± 0.76 µg/mL. This activity is close to activity of Trolox, standard used in this test, which exerted a powerful scavenging activity with IC_{50} value of 4.19 ± 0.62 µg/mL. The Aq.E extract also exerted anti-radicalar activity with IC_{50}= 9.328 ± 0.97 µg/mL, this results is in accordance with results obtained by Bouratoua et *al.* (2016) who reported that *H. tomentosum* aerial part's butanolic extract shows a potent DPPH• scavenging with IC_{50} = 14 µg/mL. The abundance of flavonoids in this plant, which are largely in charge of radical scavenging either by electron or hydrogen donation, helps to explain its powerful scavenging activity (Zhang and Tsao, 2016).

II. 2. Reducing power

The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers. The reducing power assay is frequently used to assess the natural antioxidant's capacity to give an electron (Ameni et *al.*, 2015). In this assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. In this wavelength, increasing absorbance indicates an increase in reductive ability (Fathi and Ebrahimzadeh, 2013). **Figure 16** shows the dose-response curves for the reducing power of Aq.E, Met.E and BHT. Reducing power of samples increased significantly (p<0.05) with the increase of its concentrations. *H. tomentosum* aqueous and methanolic extracts exercise a powerful reducing ability with EC_{50} value of 21.93 ± 0.01 µg/mL and 17.39 ± 0.02 µg/mL, respectively, these values are closely of EC_{50} of BHT (14.26 ± 0.02 µg/mL), a reference's antioxidant. The reductive capability is explained by the richness of both extracts by polyphenols and flavonoids. Indeed, study carried by Rebaya and Belghith (2016) highlighted a relationship between content of extract by polyphenols and flavonoids and its

reductive ability, this correlation study explain the more powerful reducing ability of Met.E than Aq.E.

II. 3. Hydrogen peroxide scavenging activity

One of the important ROS that easily penetrates biological membranes is hydrogen peroxide. Due to the possibility of subsequent conversion into other ROS such the hydroxyl radical, it can be harmful to the cells (Muthu et *al.*, 2014). **Figure 16** demonstrates that both Aq.E and Met.E of *H. tomentosum* exerted a considerable (p<0.05) and dose dependent hydrogen peroxide scavenging activity with IC_{50} value of 3.13 ± 0.49 µg/mL and 2.07 ± 0.32 µg/mL, respectively. This activity is close to that obtained by standard ascorbic acid (IC_{50}=1.23 ± 0.09 µg/mL). The potential scavenging activity is distributed to the hydroxyl groups in the aromatic ring of the phenolic components which can act as proton donor thus it neutralizes the ROS (Farahmandfar et *al.*, 2019). It donates the hydrogen to the hydrogen peroxide and cleaves it into water molecules (Prahadeesh et *al.*, 2018). Additionally, quercitrin, a significant flavonoid included in both extracts, was also revealed to have substantial H_2O_2 scavenging action (Ginting et *al.*, 2020).

II. 4. Hydroxyl radical scavenging activity

Among the reactive oxygen species, hydroxyl radical is the most reactive and predominant radical generated endogenously during aerobic metabolism to initiate cell damage *in vivo* (Kim et *al.*, 2011). It induces large aggression to human health due to its ultra-high reactivity with almost all type of biomolecules including amino acids, sugars and lipids (Engwa, 2018).It can damage the bases of DNA and mediate redox alteration of cell membrane (Ren et *al.*, 2019; Wang et *al.*, 2019). In this study, the hydroxyl radical scavenging capacity was evaluated using the Fenton reaction. From **figure 16**, it conclude that Aq.E and Met.E exhibited concentration-dependent inhibition on hydroxyl radical with IC_{50} values of 338.8 ± 2.530 µg/mL and 377.6 ± 2.57 µg/mL, respectively. On the other hand, ascorbic acid that is used as standard showed a powerful hydroxyl radical scavenging activity with IC_{50} = 290.4 ± 2.4 µg/mL. There is no significant difference between the scavenging activity of both extracts and the activity of ascorbic acid. This potent action of extracts is attributed to flavonoids which are probably the most important natural phenolic compounds and this property of radical scavenging plays a protective role in diseases (Jomova et *al.*, 2017).

Other plant in same genus as *Hypericum androsaemum* and *Hypericum lydium* showed a same hydroxyl radical scavenging activity (Valentão et *al.*, 2002; Şerbetçi et *al.*, 2012).

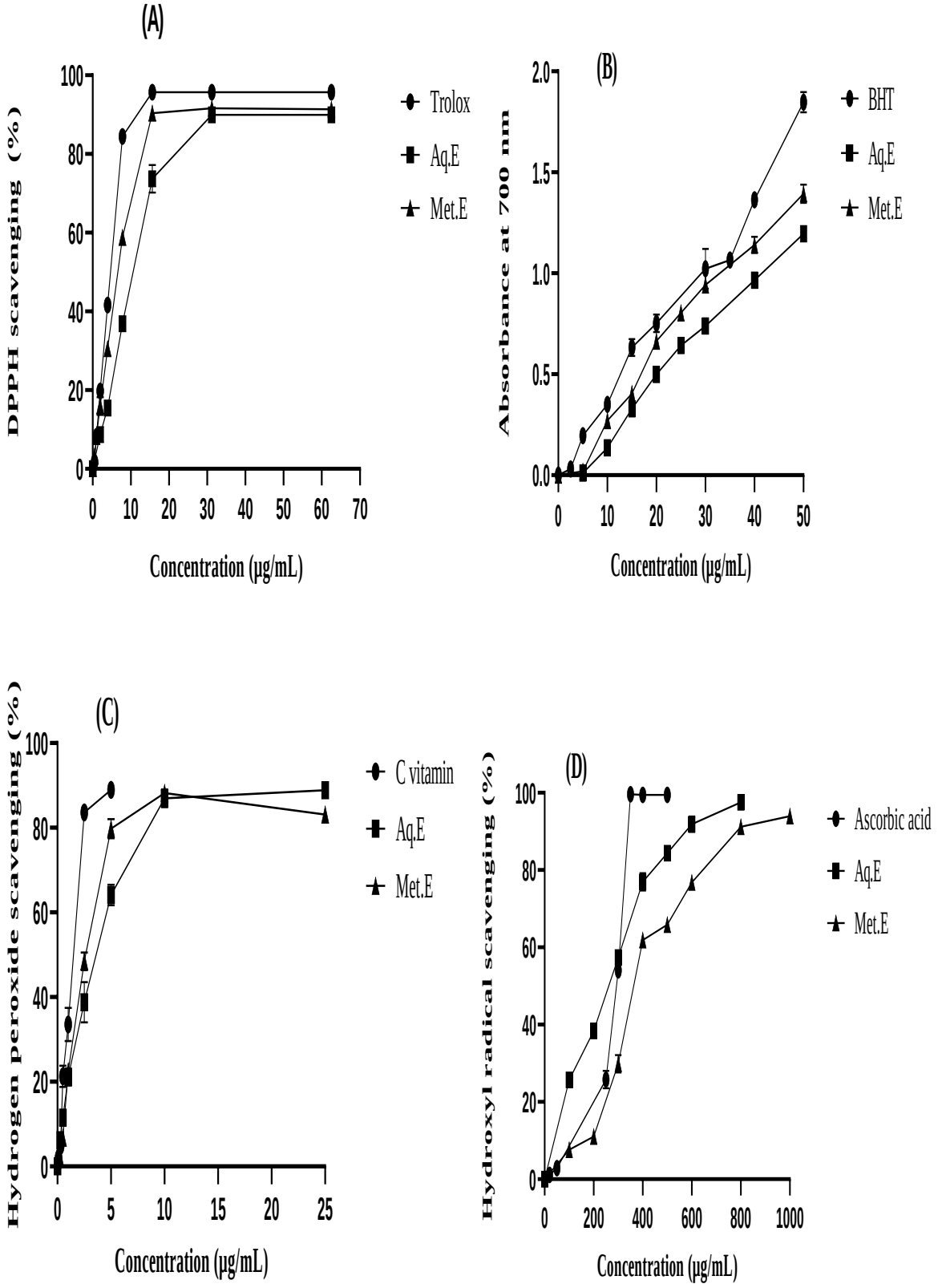

Figure 16. Antioxidant activity of Aq.E and Met.E of *H.tomentosum*. (A) DPPH• scavenging activity; (B) reducing power; (C) H_2O_2 scavenging activity; (D) OH• scavenging activity. Data represent means ± SD (n=3).

II. 5. Anti-hemolytic activity

Erythrocytes are perfect targets for ROS due to the high membrane concentration of polyunsaturated fatty acids, oxidative hemolysis in erythrocyte membranes makes for an excellent model to study antioxidant activity (Garca-Becerra et *al*., 2016; Gonçalves et *al*., 2017). The basic idea behind this test is to subject erythrocytes to a thermo-controlled free radical aggression, since the breakdown of AAPH results in the formation of molecular nitrogen and alkyl radicals. These later react with molecular oxygen to produce peroxyl radicals, which primarily attack the membranes of erythrocytes and infiltrate the cells to cause hemolysis (Elizondo-Luévano et *al.*, 2021). This hemolysis was followed continuously at 630 nm to verify changes in the turbidity of erythrocytes.

Figure 17 shows that erythrocytes cultured without addition of AAPH were very stable with limited hemolysis observed for up to 4 hours, and when they were cultured with AAPH the thermal free radical resulting attack the membrane and cause hemolysis in 2 hours. The presence of Aq.E and Met.E delays erythrocytes' hemolysis in dose dependent manner leading to resistance of red blood cells to radicalar attack. Effectively, the values of half- time hemolysis (HT_{50}), which corresponds to the necessary time to the hemolysis of half initial erythrocytes, reveal significantly (p<0.001) extension of hemolysis time compared with control (**table 5**). The Aq.E and Met.E at concentration of 50 µg/mL exhibited a best antihemolytic activity with HT_{50} of 232.75 ± 4.39 min and 218.87 ± 3.52 min, respectively. These values are close to HT_{50} of Trolox (235.35 ± 3.33 min) at the same concentration.

The effectiveness of *H.tomentosum* to prevent erythrocytes against hemolysis could be attributed to its high flavonoids contents which enhance erythrocytes' resistance against free radical species, mainly by their capacity to capture them, by donating electrons and quenching radicals formed in the aqueous phase before they can damage the erythrocytes' membrane (Gonçalves et *al.*, 2017). Indeed, epicatechin, which present with high amount in both extracts exerted a potent antihemolytic activity (Xu et *al.*, 2021). It is able to prolong the time of hemolysis to spread out in the core of the bilayer, but might also interact with its external part. It stabilize the membrane through a decrease in lipid fluidity, blocking the access of the $ROO^{\bullet}$ to the interior of erythrocytes membranes (Grzesik et *al.*, 2018).

This results are agree with results of Eslami et *al.* (2011), who reported that *Hypericum scabrum*, member of the same family of this plant, inhibited hemolysis of erythrocytes rat induced by radicalar attack.

Table 5. The protective effect of Aq.E, Met.E and Trolox against AAPH induced hemolysis of mice erythrocytes. Each value are mean ± SD (n=3).***p<0.001 vs control.

sample	Half-time hemolysis (min)			
	5 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL
control	106.53 ± 1.21			
Aq.E	132.6 ± 3.23***	142.22 ± 1.46***	167.16 ± 1.19***	232.75 ± 4.39***
Met.E	134.3 ± 4.48***	147.43 ± 3.53***	164.65 ± 3.53***	218.87± 3.53***
Trolox	-	-	185.88 ± 11.57***	235.35 ± 3.33***

II. 6. Inhibition of β-carotene bleaching

One quite popular method to monitor oxidation, is the β-carotene bleaching assay. It is based on the disappearance of the typical yellow color of β -carotene when it is co-oxidized with linoleic acid in an emulsion (Amorati and Valgimigli, 2018). The **figure 18** shows that in the presence of Aq.E or BHT the absorbance at 470 nm almost stains stable all the time of incubation, since they exerted a potent β-carotene bleaching inhibition (89.45 ± 3.3% and 77.48 ± 1.88% of inhibition in the presence of BHT and Aq.E, respectively), compared with Met.E which exerted less inhibition effect (29.43 ± 0.86% of inhibition).

The capacity of extracts to prevent or delay β-carotene bleaching could be explained by the competitively reaction of antioxidant present in both extracts with peroxyl radical generated from oxidation of linoleic acid in the presence of ROS and O_2 (Xiao et *al.*, 2020) and/or neutralize the ROS in the system (Amraoui et *al.*, 2022).

These results indicate a no corrtelation between antioxidant activity and the amount of polyphenols and flavonoids. Likely, the inhibition of β-carotene bleaching by extracts is due to other compounds as terpenes, which present with high amount in this plant (Rouis et *al.*, 2012). Indeed, several investigations revealed the *in vitro* antioxidant activity of monoterpenes (α-terpinene) and diterpenes (phytol) (Bonesi et *al.*, 2011).

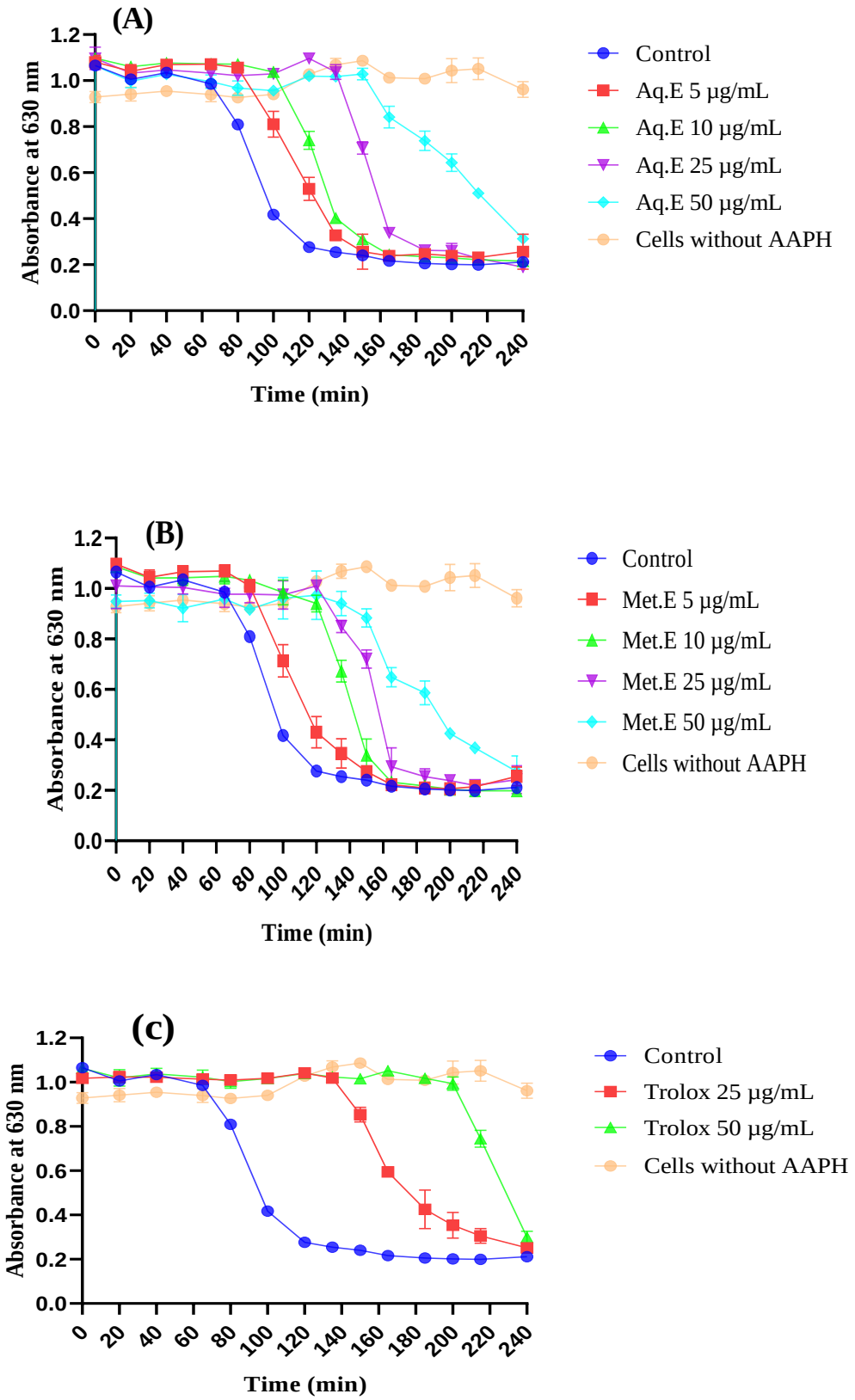

Figure 17. Changes in turbidity of erythrocytes samples during 4 hours of incubation in presence or absence of Aq.E (A), Met.E (B) and Trolox (C). Values are means ± SD (n = 3).

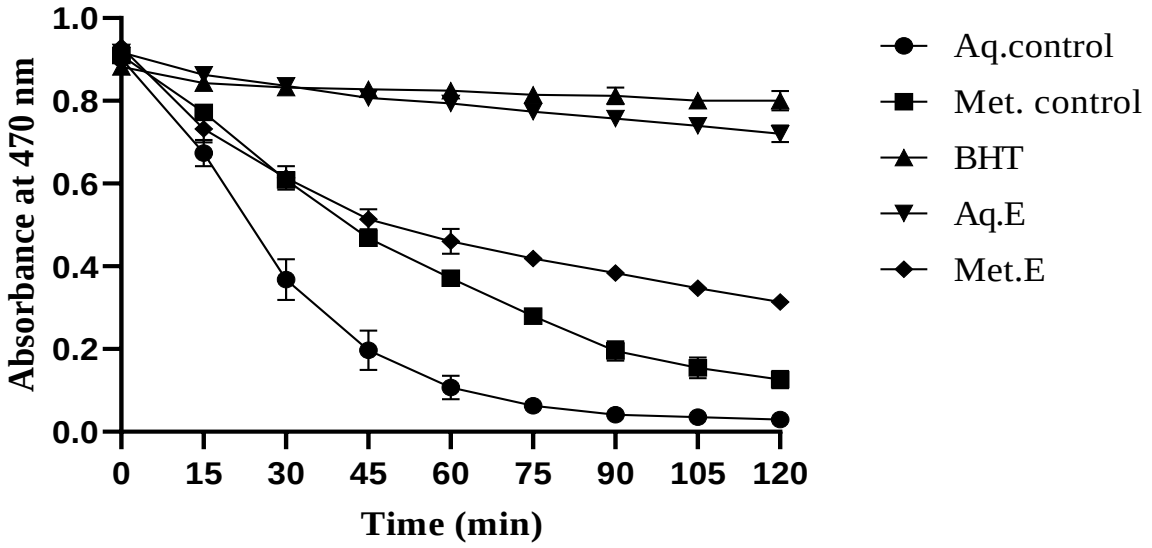

Figure 18. Inhibition of β-carotene bleaching in the presence of Aq.E and Met.E of *H. tomentosum*. Data are expressed as mean ± SD (n=3).

II. 7. Ferrous ion chelating activity

Among preventive antioxidants, metal chelating chemicals may be the most prevalent. They work by chelating transition metal ions with high affinity in order to prevent the Fenton reaction, which reduces the thermodynamic advantages of recycling transition metal ions by reducing agents and damages their catalytic redox cycle (Amorati and Valgimigli, 2018). The results show that Aq.E and Met.E inhibit the formation of Fe^{2+} - ferrozine complex in a dose dependent manner (**figure 19**) with IC_{50} of 1.3 ± 0.35 mg/mL and 1.68 ± 0.05 mg/mL, respectively. Therefore, both extracts were statistically less potent ferrous ion chelating activity (p<0.001) than EDTA (IC_{50}=0.0078 ± 0.00004 mg/mL).The same weak chelating activity was obtained with *H. perforatum* (Fathi and Ebrahimzadeh, 2013). The low activity of Aq.E and Met.E may be explained by the poverty of extracts' molecules to suitable groups that could chelate the cations. Effectively, the capacity of phenolic compounds to chelate metal ions depends on the availability of properly oriented functional groups. Although sample have a high content of polyphenols might be not chelate metal if the polyphenols present did not have suitable groups that could chelate the cations (Wong et *al.*, 2006). Indeed, Li et *al.* (2016) reported that quercitrin, which presents with high amount in Aq.E and Met.E, has only a planar Fe-binding interaction between the 4- and 5-positions with no similar steric chelation interaction.

According to Khokhar and Owusu Apenten (2003), two mechanisms are commonly proposed to explain the antioxidant role of phenolic compounds; these are metal chelation and/ or free radical scavenging. From these results it's conclude that antioxidant activity of phenolic components from *H.tomentosum* is due their free radical scavenging capacity rather than metal chelating activity.

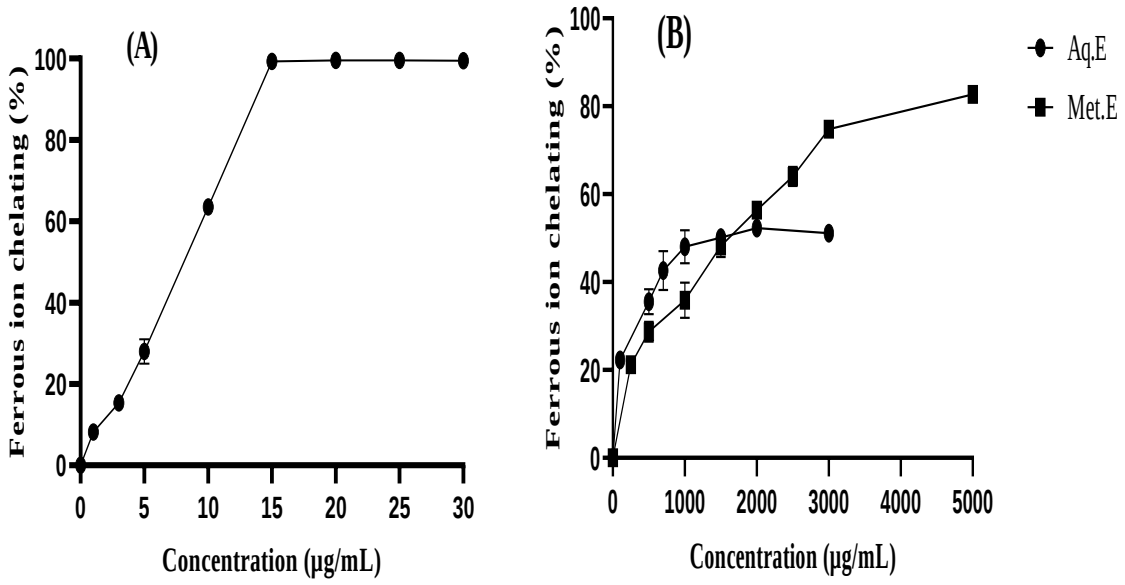

Figure 19. Ferrous ion chelating activity of EDTA (A) and *H. tomentosum* extracts' (B). The results are expressed as means ± SD (n=3).

III. The anti-inflammatory activity

III. 1. *In vitro* anti-inflammatory activity

III. 1. 1. Cytotoxicity of extracts on neutrophils

To ensure that plant extracts may be used safely, their acute toxicity must be evaluated on neutrophils. According to an experiment based on trypan blue exclusion, dead cells ingest the dye and turn blue as a result of ruptured plasma membranes. (Kernouf et *al.*, 2018).

Results show that neutrophils exposed to Met.E and Aq.E (25-200 µg/mL) for 30 min did not cause a significant reduction in cellular viability compared to the response of the untreated group (control) (**figure 20**).

This finding is supported by Schepetkin et *al.* (2020), who found that *H. perforatum* essential oil didn't effect humaun neutrophils cell viability during 30 min of incubation.

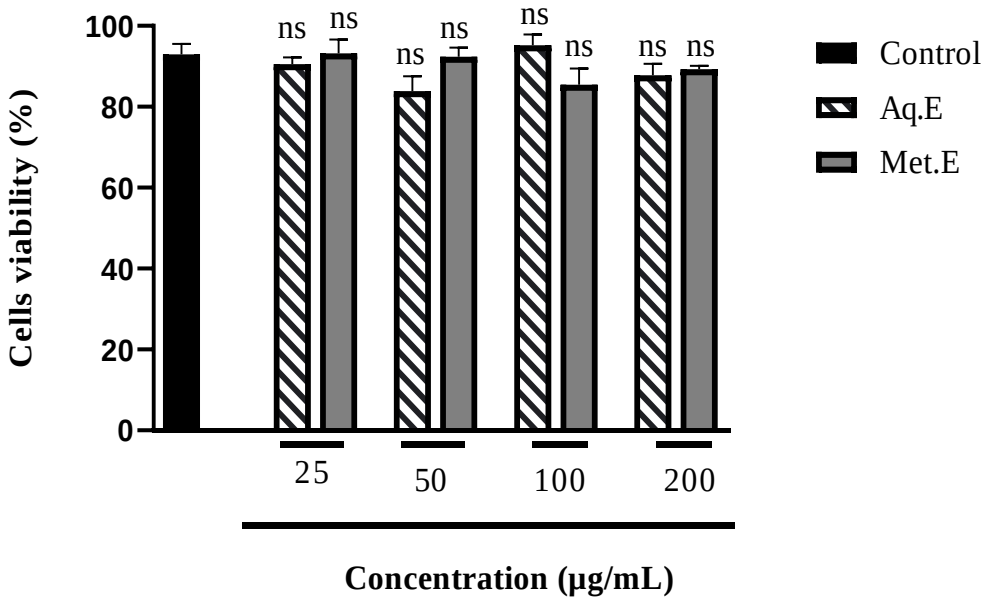

Figure 20. Neutrophils viability in the presence of Aq.E and Met.E. Data are shown as mean ± SEM (n=3).

III. 1. 2. The effect of *H. tomentosum* on elastase activity

Elastase is a serine protease belonging to the chymotrypsine family, it is deposited as active enzyme within azurophile granule of PMNs.The imbalance between elastase activity and its endogenous inhibitors can cause different illnesses due to its excessive activity and broad substrate specificity (Marinaccio et *al.*, 2022). Elastase can cause tissue damage and inflammation by breaking down extracellular matrix proteins like elastin, fibronectin and proteoglycans (Vidhya et *al.*, 2022).

Elastase released from human neutrophils was incubated with both extracts of this plant in the presence of substrate to assess the anti-inflammatory activity of *H. tomentosum.*

Both Aq.E and Met.E inhibited significantly (p<0.05) elastase activity and in dose dependent manner. The levels of inhibition at 50 µg/mL of Aq.E and Met.E were 81.67% and 85.59%, respectively (**figure 21**). The calculated IC_{50} of Aq.E and Met.E was 18.08 ± 1.25 µg/ mL and 14.32 ± 1.15 µg/ mL, respectively.

According to Piwowarski et *al.* (2011) the condensed tannins inhibit catalytic power of elastase by their properties to precipitate this enzyme. Hence, the ability to inhibit elastase is in correlation with the amount of condensed tannins present in the plant. The total condensed tannins in Aq.E and Met.E was117.29 and 163.7 µg CE/mg of extract, respectively, these

amounts of condensed tannin can clarify why Met.E is powerful than Aq.E in inhibition of elastase.

In the other hand, and According to Kwon et *al.* (2019) and Jakimiuk et *al.* (2021), phenolic chemicals including epicatechin, quercetin, quercitrin, and isoquercetrin, may be responsible for the inhibition of elastase activity. These molecules were in abundance in both Aq.E and Met.E.

Another research of Chiocchio et *al.* (2018) has shown that aerial parts of *hypericum* genus like *Hypericum perforatum*, *Hypericum hircinum* and *Hypericum scruglii* inhibit porcine pancreatic elastase.

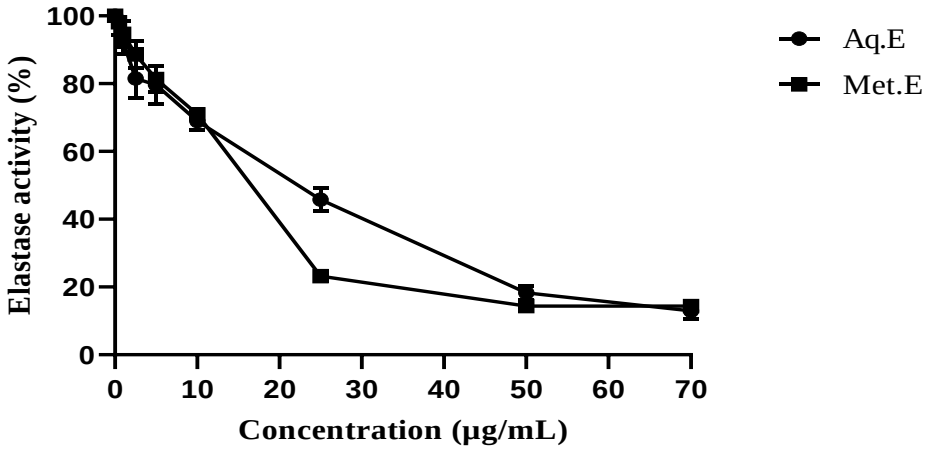

Figure 21. Effect of Aq.E and Met.E on elastase activity. The values are expressed as means ± SEM (n=3).

III. 1. 3. The effect of *H. tomentosum* on MPO activity

MPO is the most abundant protein in the primary granules of human neutrophils. It produces hypochlous acid, a strong oxidant compound, from hydrogen peroxide and chloride anions. MPO activity plays an important roles in host defense against the invasion of microorganism or other pathogens by fusion of granule containing this enzyme with phagocytic vesicle (Prokopowicz et *al.*, 2012; Buchan et *al.*, 2019). In contrast, this enzyme products modify biomolecules in hosts during excess inflammation, indicating that the action of MPO is both beneficial and harmful (Kato, 2016).

According to Tsumbu et *al.* (2012), the inhibition of neutrophils oxidant response as liberation of hypochlous acid by MPO becomes a therapeutic challenge to control or modulate an excessive inflammatory response.

The effects of extracts on MPO were evaluated by measurement the inhibition of coloration resulting after whether oxidation of TMB or oxidation of o-dianizidine by MPO. This later was obtained after induction of human PMNs degranulation by fMLP and CB. **Figure 22** shows that the pre-incubation of Aq.E and Met.E with MPO decreases significantly ($p<0.05$) its activity in presence of TMB as substrate and **figure 23** shows also that both extracts inhibit MPO activity in the presence of o-dianizidine as another substrate. From these findings, It can be assumed that some *H. tomentosum* molecules excreted a competitive inhibition with TMB for its active site and that other molecules acted as a competitive inhibition with o-dianizidine for its active site, but it is possible to speculate that there was no competition between inhibitory molecules of *H. tomentosum* and enzyme substrates.

The scavenger effects of extracts on ROS may be used to explain the decrease in MPO activity in the presence of both extracts using two distinct substrates. Indeed, it has been previously shown in antioxidant activity that both extracts have a strong hydrogen peroxide scavenging effect, which is crucial for MPO activity.

Several substances from this plant, including quercetin, gallic acid and catechin, have been shown to have strong inhibitory effects on myeloperoxidase activity (Lopes Neto et *al.*, 2020; Calassara et *al.*, 2021). Additionally, Tarasiuk et *al.* (2021) showed that chlogenic acid significantly decreased MPO activity in murine pancreas.

The values of IC_{50}, represented in **table 6**, show that Met.E is the potent inhibitor of MPO activity more than Aq.E due to the richness of Met.E by phenolic compounds. In fact, Boufadi et *al.* (2014) highlighted a relationship between the amount of polyphenols in propolis and their inhibitory activity toward MPO.

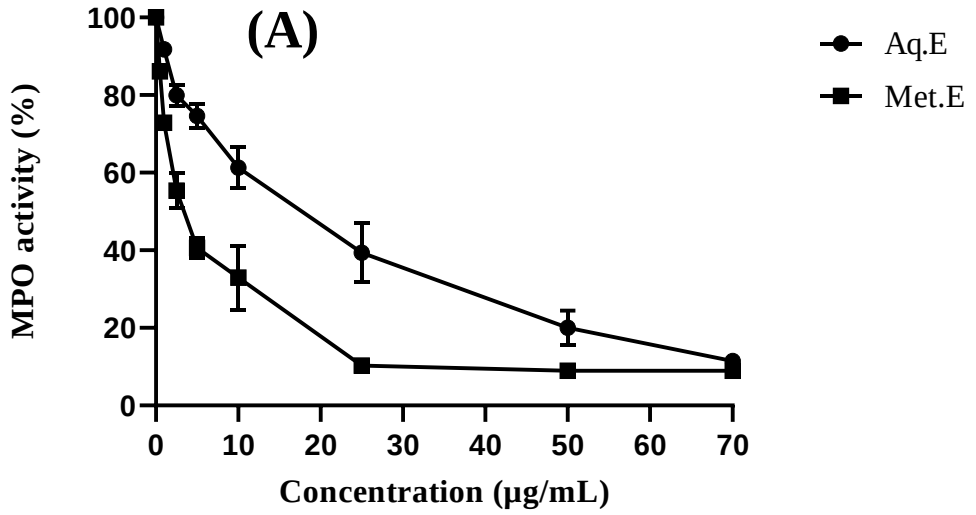

Figure 22. The effects of Aq.E and Met.E on MPO activity using TMB as substrate. The values are means ± SEM (n=3).

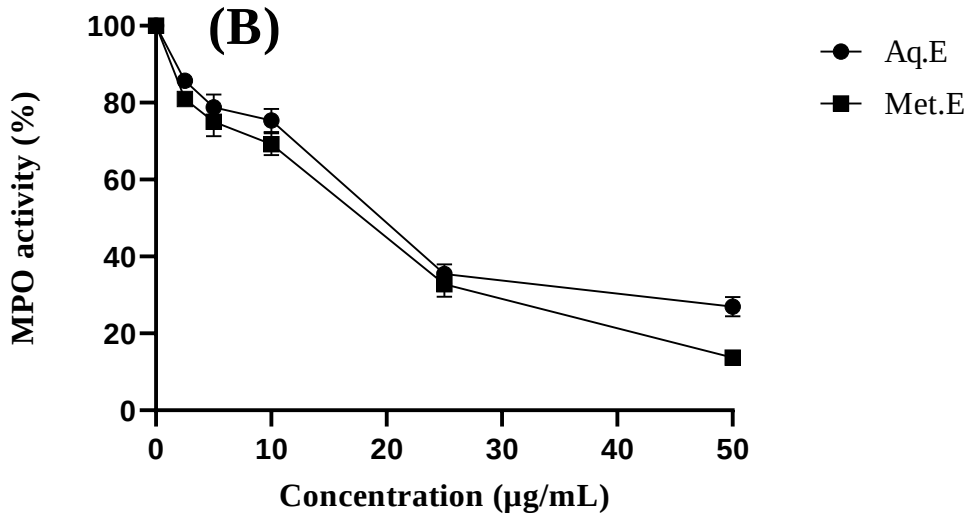

Figure 23. The effects of Aq.E and Met.E on MPO activity using o-dianizidine as substrate. The values are means ± SD (n=3).

Table 6. Value of IC_{50} of Aq.E and Met.E on MPO activitity using both TMB and o-dianizidine as substrate.

	IC_{50} (µg/mL)	
	Aq.E	Met.E
Using TMB as substrate	13,83 ± 1,14	2,65 ± 0,42
Using o-dianizidine as substrate	19,01 ± 0,53	17,84 ± 0,78

III. 1. 4. The effect on phagocytic activity

The effect of extracts on neutrophils phagocytosis function was evaluated using *C. albicans* as target of activated neutrophils. The optic microscope is used to see the phagocytic *C. albicans* (**figure 24**). The results shown in the **figure 25** indicate that, in comparison to neutrophils not treated, the phagocytosis function of neutrophils treated with both extracts decreased. The percentage of phagocytosis inhibition is 28.27%, 43.58%, 42.52% and 46.38% for four concentrations of Aq.E (5, 10, 25 and 50 µg/mL), respectively. Other inhibitions were recorded in the presence of Met.E at same concentration in order of 5.78%, 22.03%, 41.33% and 59.29% inhibition. However, these inhibitions were statistically no significant.

Phagocytosis is a fundamental mechanism for the effective elimination of disease-causing agents. It facilitates the removal of microorganisms as well as tissue debris and dead cells (Gierlikowska et *al.*, 2021). Inhibition of this function in neutrophils, which are the most important phagocytes, may compromise the body to face infections. Beside neutrophils participate in phase of resolution of inflammation by phagocytosis of tissue debris (El Kebir and Filep, 2010). Therefore, the fact that neither extracts significantly inhibited phagocytosis is a plus from a therapeutic standpoint.

These findings support those of Chen et *al.* (2014), who found that caffeic acid stimulates neutrophils' phagocytosis of *Escherichia coli*. Additionally, it has been demonstrated that quercetin increases chicken heterophil internalization and phagocytosis (Boonlaos et *al.*, 2021). Additionally, mice's alveolar macrophages' phagocytosis was boosted by chlorogenic acid (He et *al.*, 2022).

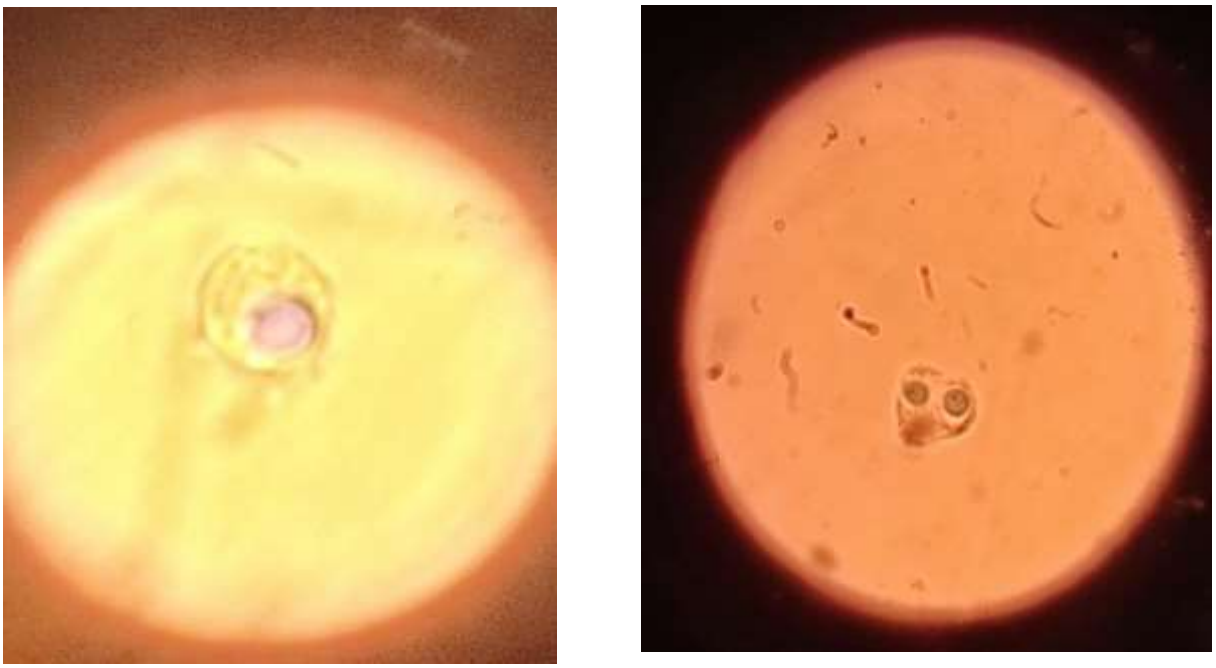

Figure 24. Photos acquired under an optical microscope demonstrate neutrophils ingesting *C.albicans.*

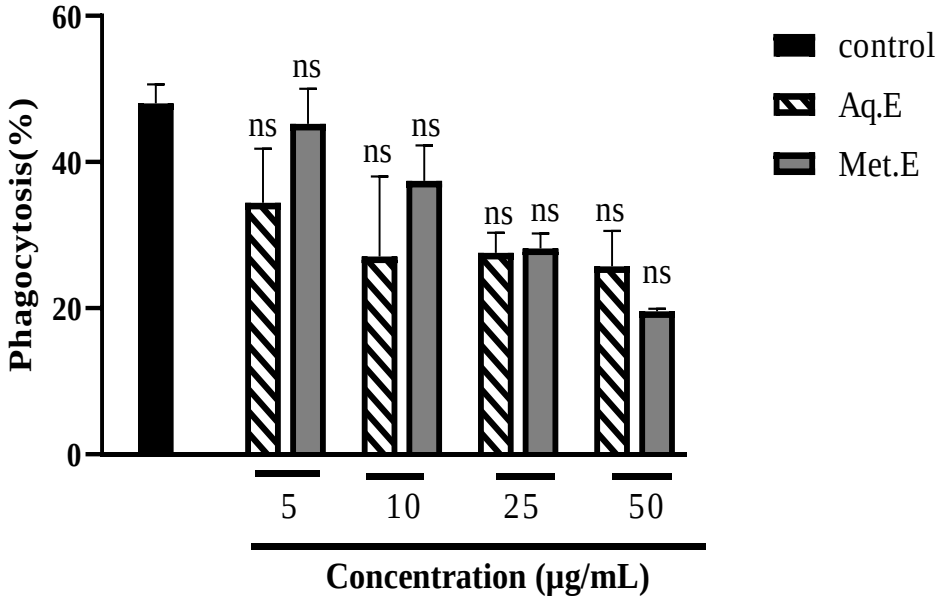

Figure 25. Percentage of phagocytosis in the presence of different concentrations of Aq.E and Met.E. The values are means ± SEM (n=3).

III. 1. 5. The effect on bactericidal activity

The main mechanism used by neutrophils to kill bacteria is bactericidal activity**,** by activation of NADPH oxidase on the phagosomal membrane to product $O_2^{\bullet-}$within the phagosome that MPO dismutates to H_2O_2. MPO then uses the H_2O_2 to oxidize chloride to hypochlous acid, which kill bacteria (Parker et *al.*, 2021). Elastase participates also in bactericidal activity by degrading outer membrane protein A, localized on the surface of Gram negative bacteria (Belaaouaj et *al.*, 2000). **Figure 26** illustrates that human neutrophils' bactericidal activity is significantly (p<0.05) inhibited by both extracts, and that *E. coli* colonies multiply as concentrations rise, the percentage of colonies arrive until 82.51 ± 4,47% and 84.1 ± 3.33% at 25 µg/mL of Aq.E and Met.E, respectively.

Based on the previously reported results, which showed that *H. tomentosum* acted as a potent inhibitor of MPO and elastase activities at the same concentrations (5, 10, and 25 g/mL) but did not significantly inhibit phagocytosis, it is possible to hypothesize that neutrophils had engulfed bacteria and that the inhibition of bactericidal was caused by the inhibition of MPO to produce hypochlous acid and prevent elastase to degrade outer membrane protein A in the surface of *E.coli*.

Other mechanisms could be able to account for the inhibition of bactericidal activity including; 1) Inhibition of phagosome-lysosome fusion to kill ingested bacteria.
2) Reduction of ROS production through respiratory burst, Demirkiran et *al.* (2013) showed that *Hypericum montbretii* has immunomodulating inhibitory activity against the oxidative burst response of human neutrophils, and Schepetkin et *al.* (2020) found that *Hypericum perforatum* essential oil reduced neutrophil ROS production. 3) Degranulation inhibition to prevent the death of no ingested *E. coli*. In fact, Bernini and Velotti (2021) showed that quercetin is a strong degranulation inhibitor.

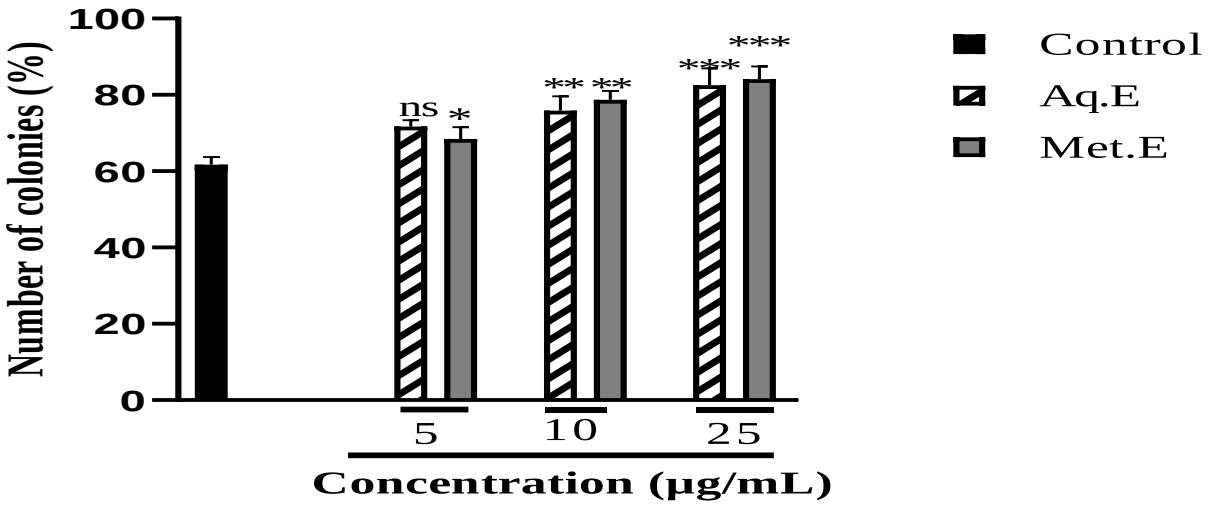

Figure 26. Effect of Aq.E and Met.E on number of bacterial colonies. The values are expressed as means ± SEM (n=3). *p<0.05. **p<0.01. ***p<0.001

III. 2. *In vivo* anti-inflammatory activity

III. 2. 1. Acute toxicity study

Acute toxicity study was conducted to determine the safe dosage of plant's extracts to animals and human subjects. In the present study, *Swiss albinos* mice were employed and administered with single dose 2 g/Kg body weight of Aq.E and Met.E from *H. tomentosum*. There are no marked behavior changes, because any signs of acute toxicity or even mortality during the 24 hours of observation as well as during the 14 days follow up period and in all tested mice administered. The same remarks were observed with mice treated with single dose of 5 g/Kg body weight. Results proved that the administration of both extracts up to the 5 g/kg dose were safe to be used for our various anti-inflammatory investigations through *in vivo* model, in a single administration. This finding is supported by Touafek et *al.* (2012b), who found that the butanolic extract from this plant can shield rat heart and renal cells from the toxicity of chemotherapy medicines.

III. 2. 2. Effect on neutrophils recruitment

The subcutaneous air pouch is an *in vivo* model used to study acute and chronic inflammation (Duarte et *al.*, 2012), injection of carrageenan solution into the pouch, as an inflammatory agent, increases leukocyte accumulation and inflammatory exudate volume in the pouch by enhancing the vascular permeability and stimulating neutrophil migration (Eteraf-Oskouei et *al.*, 2020).

Injection of carrageenan in mice's pouch induced an important PMNs' migration into site of injection (12.27 ± 0.48 million cells/ mL). However, The number of migrating neutrophils was considerably reduced in pretreated mice by both Aq.E and Met.E at 1 mg/pouch when compared to control (7.54 ± 0.75 million cells/ mL and 6.44 ± 0.42 million cells/ mL, respectively). Indomethacin at dose of 0,1 mg/ pouch was the potent inhibitor of neutrophils migration, it reduced significantly the recruitment, only 4.91 ± 0.36 million cells/ mL of PMNs were recorded after 4 hours of carrageenan injection (**figure 27**).

The richness of both extract by polyphenols and flavonoids compounds may justify this activity. Indeed, (Eteraf-Oskouei et *al.*, 2020) demonstrated that the presence of flavonoids in the bee pollen methanolic extract inhibited leukocyte accumulation. Other researches have linked this function to bioactive substances found in plant extracts, it has reported that chlorogenic acid and caffeic acid reduce leukocytes migration (Furtado et *al.*, 2016; Torres-Rêgo et *al.*, 2016). Additionally, Cheng et *al.* (2016) demonstrated that orally administration of epicathechin attenuated neutrophils infiltration.

Based on the mechanism by which carrageenan causes inflammation, it is possible to hypothesize that the inhibition of neutrophil migration is caused by the suppression of the production of pro-inflammatory cytokines, which are in charge of producing the adhesive molecules required for neutrophil migration (Borish and Steinke, 2003). Indeed, Šavikin et *al.*, (2006) showed that hypericin**,**a napthtodiotrone presenet in this genus (Henzelyová and Čellárová, 2018), substantially suppressed IL-1 and IL-6 transcription. Another study conducted by Ling et *al.* (2020) has shown that the main constituents of both extracts, quercitrin and isoquercitrin, exerted a notable inhibition of IL-6 secretion.

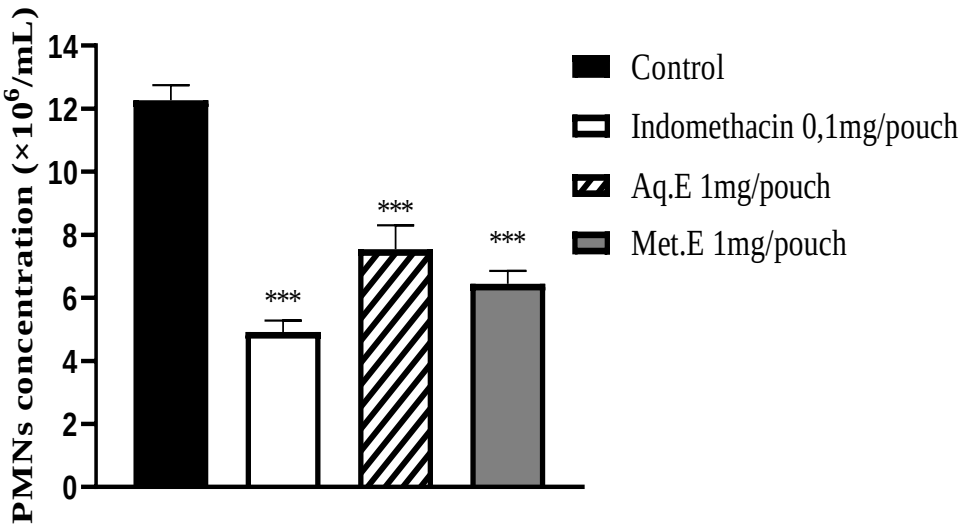

Figure 27. Effect of indomethacin, Aq.E and Met.E on neutrophils concentration in the pouch of mice. Data are expressed as means ± SEM (n = 6). (p<0.001).

III. 2. 3. Croton oil induced ear edema

Croton oil, is a constituent of *Croton tiglium* plant, containing phorbol ester with TPA (12-O-tetradecanoyl-13-phorbol acetate) predominantly as active compounds. Induction of acute inflammation by croton oil occurs by increase vascular permeability, vasodilation and swelling due to the release of histamine and serotonin, followed by activating phospholipase A2 which releases arachidonic acid from the cell membrane which in turn metabolism to prostaglandin by cyclooxygenase and to leukotriene by 5-lypoxygenase (Puspawati and Rita, 2019; Sangchart et *al.*, 2021).

Treatment by croton oil topically to mice ear promoted an edema as signed by the increase in right ear thickness. The effect of topical application of extracts and indomethacin on croton oil induced ear edema was summarized in **figure 28**. The extracts and indomethacin reduced significantly (p<0.001) mice ear edema volumes, measured as ear thickness; the application of 2 mg/ ear of *H. tomentosum* Aq. E and Met.E inhibited 68.75% and 67%, respectively mice ear edema at 6 hours after croton oil application. Hence, both extracts exerted a nearly inhibition. While, indomethacin 0.5 mg/ ear, a non-selective inhibitor of COX, reduces the production of prostaglandins as was expected, was the most effective to prevent mouse ear edema with inhibition of 78.75%.

This potent activity of Aq.E and Met.E to prevent ear edema may be contribute to their compounds. In fact, according to Bouratoua et *al.* (2016), this plant contains phytosterols which prevent phorbol-induced edema (Conforti et *al.*, 2008). In the other hand, a previous report

described the edema inhibition by topical application of *H. reflexum* (Sánchez-Mateo et *al.*, 2006). Additionally, it has reported that topical application of *H. perforatum* exerted a potent anti-inflammatory activity by inhibition of edema induced by croton oil (Sosa et *al.*, 2006).

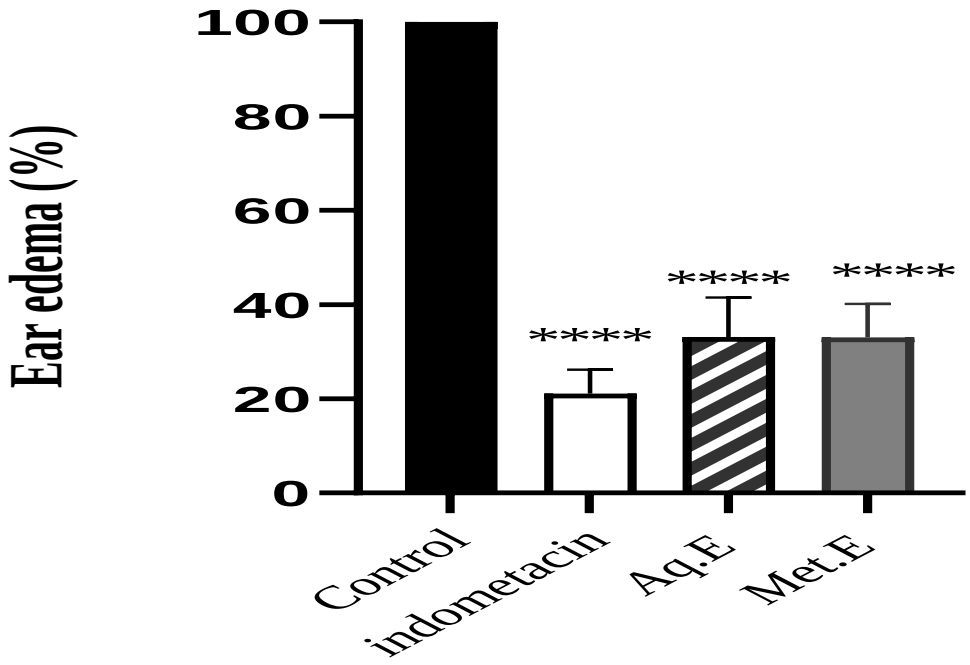

Figure 28. Ear edema in the presence of indomethacin, Aq.E and Met.E. Data are means ± SEM (n=6) (p<0.001).

III. 2. 4. Carrageenan induced paw edema

Carrageenan-induced hind paw edema model was employed to evaluate extracts' anti-inflammatory activity by giving mice a sub-plantar injection of 1% carrageenan. This model has been widely used for the discovery and evaluation of anti-inflammatory drugs (Abiye et *al.*, 2019).

The results show that Aq.E and Met.E at 200 mg/Kg and 400 mg/Kg body's weight slightly reduce edema production throughout the entire measurement period (4 hours). In contrast, indomethacin had a strong paw edema inhibition effect (**figure 29**). The findings of the weight of paw measurement after 4 hours of carrageenan injection are shown in **figure 30**, which demonstrates that Aq.E and Met.E in both dosages (200 mg/kg and 400 mg/kg) had no significant impact on paw weight. Whereas, indomethacin at a dose of 20 mg/Kg caused a significant (p<0.05) reduction in the weight of the paws, and it could prevent 79.74% of paw edema.

The inflammatory process is triggered by carrageenan in two stages. The first phase, which lasts for the first 2.5 hours after the carrageenan injection, is caused by the release of mediators that affect vascular permeability, such as serotonin, histamine, and bradykinin. (Yonathan et *al.*, 2006). The final stage, which takes place 2.5 to 6 hours after the injection of carrageenan, is characterized by an excess of prostaglandins in the tissues, and the maximum vascular response is dictated by neutrophil migration to the inflamed area. (Abiye et *al.*, 2019; Khan et *al.*, 2020).

According to previous findings, injection in pouch of *H. tomentosum* Aq.E and Met.E exerted a significant inhibition of neutrophils migration into inflammatory site when it is induced by the same phlogestic agents. Based on this finding and the mechanism of carrageenan-induced paw edema, it is possible to suggest that no significant antiedematous activity of *H. tomentosum*is due to inadequate absorption of plant's compounds from the intestine in orally administration.

When applied topically, *H. tomentosum* extracts effectively reduced edema induced by croton oil. However, when administered orally, Aq.E and Met.E were ineffective to reduce the edema caused by carrageenan. This result may be explained by the different mechanisms of action of the two phlogistic agents, as well as the fact that when taken orally, flavonoids undergo metabolite transformation (Escribano-Ferrer et *al.*, 2019). Indeed, phenolic acid is more effective in topic application according to Fernández et *al.* (1998). Additionally, Carlson et *al.* (1989) reported that the topical administration is the most effective since it allows for the massive absorption of secondary metabolic products.

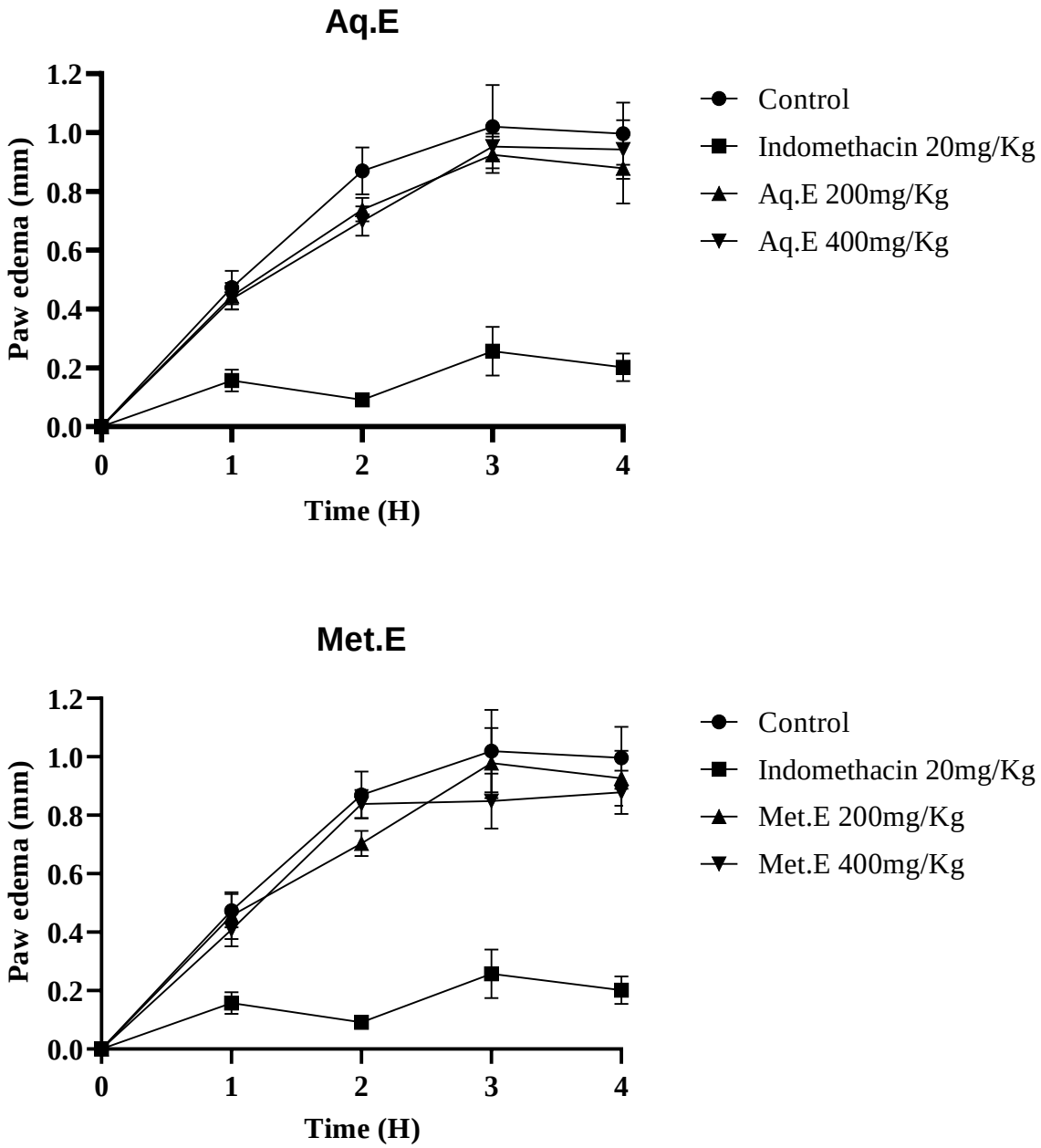

Figure 29. Kinetic of paw edema, induced by sub-plantar injection of carrageenan, in the presence of indomethacin, Aq.E and Met.E on paw edema in mice. Data are mean ± SEM (n=5).

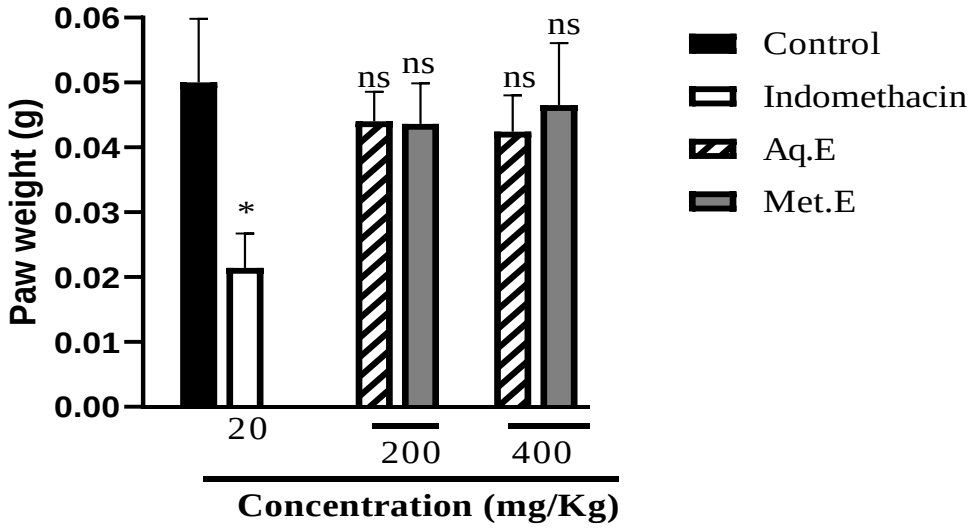

Figure 30. Effect of indomethacin, Aq.E and Met.E on paw edema induced by subplantar injection of carrageenan in mice. All data are expressed as mean ± SEM (n=5). (p<0.05)

III. 2. 5. Analgesic effect

The acetic acid-induced vascular permeability model, as an acute inflammation model, is used to evaluate the inhibitory activity of samples against pain. Acetic acid causes nociception in this test via a process that is dependent on the local cells (macrophages and mast cells) that are present in the peritoneal cavity. TNF-α, IL-1, and IL-8 are cytokines that are released by these cells and are involved in the contortion response (Santos et *al.*, 2020).

Figure 31 shows that both extracts at 200 and 400 mg/Kg and aspirin exerted a significant (p<0.01), and dose dependent manner, protective effects vis a vis pain induced by intraperitoneal injection of acetic acid by reducing the number of contortion, the best inhibitory effects was observed with Met.E at 400 mg/Kg with 65.47% of inhibition followed by Aq.E at same concentration with inhibition of 48.37% , these activities was good than aspirin at 100 mg/kg which reduces 44.9% contortion's number.

Acetic acid, according to Abiye et *al*. (2019), irritates the peritoneal cavity, stimulating local nociceptors and inducing the release of endogenous chemicals from peripheral sensory nerve endings, including histamine, prostaglandins, bradykinins, and substance P. This shows that the extract of *H. tomentosum* may exert its peripheral analgesic effects by inhibiting the release of these endogenous chemicals and inflammatory mediators.

The analgesic activity of both extracts may be associated with their antioxidant properties demonstrated by *in vitro* methods used. Indeed, Santos et *al.* (2013) reported that oxidative stress has a crucial role in signaling of nociception and is involved in the process of pain.

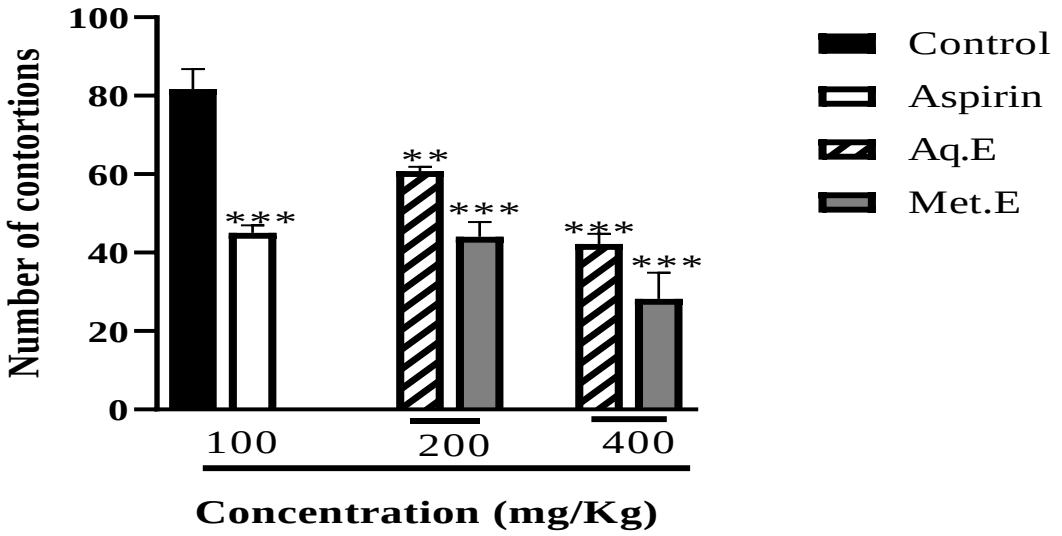

Figure 31. Effect of aspirin, Aq.E and Met.E on number of contortion induced by intraperitoneal injection of acetic acid in mice. Data are expressed as means ± SEM (n=6). ** $p<0.01$. *** $p<0.001$.

CONCLUSION AND PERSPECTIVES

Conclusion and perspectives

The main purpose of this work is to use scientific basis to verify some anti-inflammatory and antioxidant properties attribute to *Hypericum tomentosum* plant extracts.

The current study revealed the richness of *H.tomentosum* leaves aqueous and methanolic extracts by polyphenols, flavonoids and tannins, besides, an analyze using HPLC allowed to identify ten acids, fourteen flavonoids one biflavonoid and two phenyl aldehydes on these extracts.

The hydro-methanolic mixture proved to be the best solvent for extracting phenolic compounds, thanks to its ability to extract polar and apolar molecules, which resulted in the good yields obtained with this solvent.

In the study of the *in vitro* antioxidant activity of these two extracts, the scavenging capacity of $DPPH^{\cdot}$, hydroxyl radical and hydrogen peroxide was targeted. Considerable antioxidant potential was shown in these tests by the two extracts studied. In addition, both extracts were found to have ferric ion -reducing ability and exert protective effect against lipid peroxidation in AAPH-induced erythrocyte hemolysis model and β-carotene bleaching model. However, the extracts studied show a weak ferrous ion chelating activity.

Cytotoxic effect of studied extracts was tested on human neutrophils cells, where both aqueous and methanolic extracts didn't cause cells death. The toxicity effect was tested also using mice, where no mortality was recorded.

The anti-inflammatory activity has been confirmed by tests to evaluate the effects of the studied extracts on certain functions of human neutrophils. The two extracts showed significant dose-dependent inhibition on neutrophil elastase, MPO and bactericidal activities. However, the effect of the extracts on the phagocytosis of *Candida albicans* by neutrophils was minimal and not significant.

Both Extracts of *H. tomentosum* exert significant anti-edematous effects when applied topically. However, low activity was recorded in the case of oral administration. These extracts also inhibit the recruitment of immune cells to the inflammatory site. On the other hand, both extracts have analgesic properties, thus justifying their traditional use.

The results of the present study show that the aqueous and methanolic extracts of this plant possess antioxidant and anti-inflammatory effects. This medicinal plant could be considered as an interesting source of antioxidant and anti-inflammatory agents with potential pharmacological applications in pathologies. However, this study requires further investigations concerning in particular:

- ✓ Expand the research scope of other mediators and tests, whether *in vitro* or *in vivo*.
- ✓ Isolation and purification of the molecules responsible for the various activities obtained by these extracts and understanding the mechanisms at the cellular and molecular level, in the hope of obtaining new molecules with various effects on the various effectors of inflammation.
- ✓ The in-depth study on the pharmacokinetics and pharmacodynamics of the active components in these extracts is very important for determining the preventive and therapeutic dosage.

REFERENCES

References bibliographic

Abdulkhaleq, L.A., Assi, M.A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y.H., Hezmee, M.N.M., 2018. The crucial roles of inflammatory mediators in inflammation: A review. *Vet. World* 11, 627–635.

Abiye, A.A., Tadesse, W.T., Assefa, S., Hailu, A.E., 2019. Evaluation of Analgesic and Anti-Inflammatory Activities of hydro-alcoholic leaf extract of *Hypericum* revolutum in Mice. *Int. J. Biomed. Res.*10 (3).

Albano, S.M., Miguel, M.G., 2011. Biological activities of extracts of plants grown in Portug*al.Ind. Crops Prod*. 33, 338–343.

Aldini, G., Altomare, A., Baron, G., Vistoli, G., Carini, M., Borsani, L., Sergio, F., 2018. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic. Res.* 52, 751–762.

Altomare, A., Baron, G., Brioschi, M., Longoni, M., Butti, R., Valvassori, E., Tremoli, E., Carini, M., Agostoni, P., Vistoli, G., Banfi, C., Aldini, G., 2020. N-Acetyl-Cysteine regenerates albumin Cys34 by a thiol-disulfide breaking mechanism: an explanation of its extracellular antioxidant activity. *Antioxidants* 9, 367.

Ameni, D., Baghiani, A., Boumerfeg, S., Dahamna, S., Khennouf, S., Zarga, M.H.A., Arrar, L., 2015. Phytochemical profiles, antioxidant capacity and protective effect against AAPH-induced mouse erythrocyte damage by *Daphne gnidium L.* Shoots Extracts. *Int. J. Pharm. Pharm.* Sci. 7, 9.

Amorati, R., Valgimigli, L., 2018. Methods to measure the antioxidant activity of phytochemicals and plant extracts. *J. Agric. Food Chem.* 66, 3324–3329.

Amraoui, N., Laroui, H., Charef, N.E., Baghiani, A., Arrar, L., 2022. Antioxidant, anti-hemolytic and anti-inflammatory activities of aqueous extract of aerial parts of *Rosmarinus tournefortii*. *Annals of R.S.C.B.* 26, 2912 - 2923

Amroun, D., Hamoudi, M., Khennouf, S., Boutefnouchet, S., Harzallah, D., Amrane, M., Dahamna, S., 2021. In-vivo anti-inflammatory activity and safety assessment of the aqueous extract of Algerian *Erica arborea L.* (Ericaceae) aerial parts. *J. Ethnopharmacol.* 271, 113881.

Arulselvan, P., Fard, M.T., Tan, W.S., Gothai, S., Fakurazi, S., Norhaizan, M.E., Kumar, S.S., 2016. Role of antioxidants and natural products in inflammation. *Oxid. Med. Cell. Longev.* 2016, 1–15.

Asan, H.S., 2019. The studies on the endemic *Hypericum* species from Turkey. *J. Life Sci. Res*.19, 253-268.

Asan, S.H., 2021. Phenolic compound contents of *Hypericum* species from Turkey, in: propagation and genetic manipulation of plants. Iram Siddique, india, pp. 43–68.

Azahar, N.F., Gani, S.S.A., Mohd Mokhtar, N.F., 2017. Optimization of phenolics and flavonoids extraction conditions of *Curcuma Zedoaria* leaves using response surface methodology.*Chem. Cent. J.* 11, 96.

Bahorun, T., Gressier, B., Trotin, F., Brunete, C., Dine, T., Vasseur, J., Grazin J.C., Pinkas, M., Luycky, M., Grazin,M., 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical prepration. *Arzneimittel Forschung*, 46, 1086-1089.

Barnig, C., 2016. Médiateurs lipidiques pro-résolvant dans l'inflammation allergique. *Rev. Fr. Allergol.* 56, 38–42.

Baudin, B., 2020. Marqueurs d'oxydation des acides nucléiques. Rev. *Francoph. Lab.* 2020, 56–61.

Bernini, R., Velotti, F., 2021. Natural polyphenols as immunomodulators to rescue immune response homeostasis: quercetin as a research model against severe COVID-19. *ISO4.* 26, 5803.

Bernstein, N., Akram, M., Daniyal, M., Koltai, H., Fridlender, M., Gorelick, J., 2018. Antiinflammatory potential of medicinal plants: a source for therapeutic secondary metabolites, in: Advances in Agronomy. Elsevier, pp. 131–183.

Bonesi, M., Okusa, P.N., Tundis, R., Loizzo, M.R., Menichini, Federica, Stévigny, C., Duez, P., Menichini, Francesco, 2011. Chemical composition, antioxidant Properties and anti-cholinesterase activity of *Cordia gilletii* (Boraginaceae) leaves essential oil. *Nat. Prod. Commun.* 6

Bonniaud, P., Beltramo, G., Foignot, C., Ornetti, P., Georges, M., 2018. Pneumopathies non infectieuses induites par les biothérapies ciblées. *Rev. Rhum. Monogr*. 85, 184–190.

Boonlaos, A., Wechsirisan, W., Chaibuth, P., Chupia, V., Chotinun, S., Chuammitri, P., 2021. Quercetin enhances and modulates the fungal killing efficacy of chicken heterophils through immunological recognition, effector functions, and resolution. *Comp. Immunol. Microbiol. Infect. Dis.* 74, 101582.

Borish, L.C., Steinke, J.W., 2003. 2. Cytokines and chemokines. *J. Allergy Clin. Immunol.* 111, S460–S475.

Boufadi, Y., Soubhye, J., Riazi, A., Rousseau, A., Vanhaeverbeek, M., Nève, J., Boudjeltia, K., Van Antwerpen, P., 2014. Characterization and antioxidant properties of six Algerian propolis extracts: ethyl acetate extracts inhibit myeloperoxidase activity. *Int. J. Mol. Sci.* 15, 2327–2345.

Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., Nasri, M., 2009. Antioxidant and free radical-scavenging activities of smooth hound (Mustelus mustelus) muscle protein hydrolysates obtained by gastrointestinal proteases. *Food Chem*. 114, 1198–1205.

Bouratoua, A., Touafek, O., Kabouche, A., Zerizer, S., Kabouche, Z., 2016. Components and antioxidant and immunomodulatory activities of *Hypericum tomentosum* subsp. pubescens. *Chem. Nat. Compd.* 52, 893–895.

Bouriche, H., Kada, S., Senator, A., demirtas, I., Ozen, T., Toptanci, B.Ç., Kizil, G., Kizil, M., 2017. Phenolic content and biomolecule oxidation protective activity of *Globularia alypum* extracts. *Braz. Arch. Biol. Technol.* 60.

Bouriche, H., Saidi, A., Ferradji, A., Belambri, S., Senator, A., 2016. Anti-inflammatory and immunomodulatory properties of Pistacia lentiscus extracts. *J. Appl. Pharm. Sci.* 140–146.

Boussif, A., Rolas, L., Weiss, E., Bouriche, H., Moreau, R., Périanin, A., 2016. Impaired intracellular signaling, myeloperoxidase release and bactericidal activity of neutrophils from patients with alcoholic cirrhosis. *J. Hepatol.* 64, 1041–1048.

Boussouf, L., Boutennoune, H., Kebieche, M., Adjeroud, N., Al-Qaoud, K., Madani, K., 2017. Anti-inflammatory, analgesic and antioxidant effects of phenolic compound from Algerian Mentha rotundifolia L. leaves on experimental animals. *South Afr. J. Bot.* 113, 77–83.

Briskin, D.P., Gawienowski, M.C., 2001. Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. *Plant Physiol. Biochem.* 39, 1075–1081.

Buchan, K.D., Prajsnar, T.K., Ogryzko, N.V., de Jong, N.W.M., van Gent, M., Kolata, J., Foster, S.J., van Strijp, J.A.G., Renshaw, S.A., 2019. A transgenic zebrafish line for in vivo visualisation of neutrophil myeloperoxidase. *Plos one* 14, e0215592.

Bueno, F.R., Silva, C.A., Oba, A., Almeida, M., Medeiros, L.G., Pitarello, A.S., Shinyashike, A.T., 2020. Performance, carcass yield, and meat quality of broilers supplemented with organic or inorganic zinc. *Arq. Bras. Med. Veterinária E Zootec.* 72, 224–232.

Butterfield, D.A., Halliwell, B., 2019. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nat. Rev. Neurosci*. 20, 148–160.

Calassara, L.L., Pinto, S.C., Condack, C.P.M., Leite, B.F., Nery, L.C. do E.S., Tinoco, L.W., Aguiar, F.A., Leal, I.C.R., Martins, S.M., Silva, L.L. da, Raimundo, J.M., Muzitano, M.F., 2021. Isolation and characterization of flavonoids from *Tapirira guianensis*leaves with vasodilatory and myeloperoxidase-inhibitory activities. *Nat. Prod. Res*. 35, 5480–5483.

Carlson, R.P., O'Neill-Davis, L., Calhoun, W., Datko, L., Musser, J.H., Kreft, A.F., Chang, J.Y., 1989. Effect of a 5-lipoxygenase (5-LO)/cyclooxygenase (CO) inhibitor, WY-47, 288, on cutaneous models of inflammation. *Agents Actions* 26, 319–328.

Castan, L., Magnan, A., Bouchaud, G., 2016. Rôles des récepteurs de chimiokines dans les maladies allergiques. *Rev. Fr. Allergol.* 56, 426–433.

Cederbaum, A.I., 2017. Cytochrome P450 and oxidative stress in the liver, in: liver pathophysiology. Elsevier, pp. 401–419.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2018. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9, 7204–7218.

Chen, M.-L., Wu, S., Tsai, T.-C., Wang, L.-K., Chou, W.-M., Tsai, F.-M., 2014. The caffeic acid in aqueous extract of *Tournefortia sarmentosa* enhances neutrophil phagocytosis of *Escherichia coli*. *Immunopharmacol. Immunotoxicol.* 36, 390–396.

Cheynier, V., 2005. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.* 81, 223S-229S.

Chiocchio, I., Mandrone, M., Sanna, C., Maxia, A., Tacchini, M., Poli, F., 2018. Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest. *Ind. Crops Prod.* 122, 498–505.

Conforti, F., Sosa, S., Marrelli, M., Menichini, Federica, Statti, G.A., Uzunov, D., Tubaro, A., Menichini, Francesco, Loggia, R.D., 2008. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. *J. Ethnopharmacol.* 116, 144–151.

Dai, J., Mumper, R.J., 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *ISO4.* 15, 7313–7352.

Dalto, D., Matte, J.-J., 2017. Pyridoxine (Vitamin B6) and the glutathione peroxidase system; a link between one-carbon metabolism and antioxidation. *Nutr. J.* 9, 189.

Dasuri, K., Zhang, L., Keller, J.N., 2013. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radic. Biol. Med.* 62, 170–185.

Demidchik, V., 2015. Mechanisms of oxidative stress in plants: From classical chemistry to *cell biology. Environ. Exp. Bot.* 109, 212–228.

Demirkiran, O., Mesaik, M.A., Beynek, H., Abbaskhan, A., Choudhary, M.I., 2013. Immunosupressive Phenolic Constituents from *Hypericum montbretii Spach*. *Rec Nat Prod*.7 (3), 210-219

Desai, O., Winkler, J., Minasyan, M., Herzog, E.L., 2018. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. *Front. Med.* 5, 43.

Dramane, P., Yhi-pênê, N.J., Adama, H., 2019. Antioxidant and anti-inflammatory activities of *Caralluma acutangula* (decne.) N.E.Br extracts, a medicinal plant from Burkina Faso. *J. Drug Deliv. Ther.* 9, 155–161.

Drzeżdżon, J., Jacewicz, D., Chmurzyński, L., 2018. The impact of environmental contamination on the generation of reactive oxygen and nitrogen species – consequences for plants and humans. *Environ. Int.* 119, 133–151.

Duarte, D.B., Vasko, M.R., Fehrenbacher, J.C., 2012. Models of inflammation: carrageenan air pouch. *Curr. Protoc. Pharmacol.* 56, 5.6.1-5.6.8.

Durand, D., Damon, M., Gobert, M., 2013. Le stress oxydant chez les animaux de rente : principes généraux. *Cah. Nutr. Diététique* 48, 218–224.

El Kebir, D., Filep, J.G., 2010. Role of neutrophil apoptosis in the resolution of inflammation. *Sci. World J.* 10, 1731–1748.

El-Beltagi, H.S., Mohamed, H.I., 2013. Reactive oxygen species, lipid peroxidation and antioxidative defense mechanism. *Not. Bot. Horti Agrobot. Cluj-Napoca* 41, 44.

Elizondo-Luévano, J.H., Castro-Ríos, R., López-Abán, J., Gorgojo-Galindo, O., Fernández-Soto, P., Vicente, B., Muro, A., Chávez-Montes, A., 2021. Berberine: A nematocidal alkaloid from *Argemone mexicana* against Strongyloides venezuelensis. *Exp. Parasitol.* 220.

Elufioye, T.O., Olusola, D.M., Oyedeji, A.O., 2019. Correlation of total phenolic, flavonoid and tannin content of *Bryophyllum pinnatum* (*Lam.*) (Crassulaceae) extract with the antioxidant and anticholinesterase activities. *Pharmacogn. J.* 11, 1003–1009.

Engwa, G.A., 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases, in: Asao, T., Asaduzzaman, M. (Eds.), Phytochemicals - Source of Antioxidants and Role in Disease Prevention. *InTech.*

Epa, C., Penemé, B.M.L., Etou Ossibi, A.W., Agbonon, A., Attibayeba, Ongoka, P.R., Abena, A.A., 2018. Effets anti-inflammatoire, analgesique et antipyretique de L'extrait aqueux des ecorces de tronc de *Maranthes glabra.* (OLIV.) Prance (chrysobalaceae). *Ann. L'Université Mar. N'GOUABI* 18.

Escribano-Ferrer, E., Queralt Regué, J., Garcia-Sala, X., Boix Montañés, A., Lamuela-Raventos, R.M., 2019. *In Vivo* anti-inflammatory and antiallergic activity of pure naringenin, Naringenin chalcone, and quercetin in mice. *J. Nat. Prod.* 82, 177–182.

Eslami, B., Nabavi, S.F., Nabavi, S.M., Ebrahimzadeh, M.A., Mahmoudi, M., 2011. Pharmacological activities of *Hypericum scabrum L.Eur. Rev. Med. Pharmacol.*15, 532–537.

Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sánchez-Pérez, P., Cadenas, S., Lamas, S., 2015. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol.* 6, 183–197.

Eteraf-Oskouei, T., Shafiee-Khamneh, A., Heshmati-Afshar, F., Delazar, A., 2020. Anti-inflammatory and anti-angiogenesis effect of bee pollen methanolic extract using air pouch model of inflammation. *Res. Pharm. Sci*. 15, 66.

Farahmandfar, R., Esmaeilzadeh Kenari, R., Asnaashari, M., Shahrampour, D., Bakhshandeh, T., 2019. Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaves extracts as affected by various solvents and extraction methods. *Food Sci. Nutr.* 7, 465–475.

Fathi, H., Ebrahimzadeh, M.A., 2013. Antioxidant and free radical scavenging activities of *Hypericum perforatum L.* (st. John's wort).*Int. J. Forest, Soil and Erosion.* 3 (2),68-72

Favier, A., 2006. Stress oxydant et pathologies humaines. *Ann. Pharm. Fr.* 64, 390–396.

Fernández, M.A., Sáenz, M.T., García, M.D., 1998. Anti-inflammatory activity in rats and mice of phenolic acids isolated from *Scrophularia frutescens*. *J. Pharm. Pharmacol.* 50, 1183–1186.

Fleming, E., Luo, Y., 2021. Co-delivery of synergistic antioxidants from food sources for the prevention of oxidative stress. J. Agric. *Food Res.* 3, 100107.

Francenia Santos-Sánchez, N., Salas-Coronado, R., Villanueva-Cañongo, C., Hernández-Carlos, B., 2019. Antioxidant compounds and their antioxidant mechanism, in: shalaby, E. (Ed.), Antioxidants. IntechOpen.

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D.W., Fasano, A., Miller, G.W., Miller, A.H., Mantovani, A., Weyand, C.M., Barzilai, N., Goronzy, J.J., Rando, T.A., Effros, R.B., Lucia, A., Kleinstreuer, N., Slavich, G.M., 2019. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 25, 1822–1832.

Furtado, A.A., Torres-Rêgo, M., Lima, M.C.J.S., Bitencourt, M.A.O., Estrela, A.B., Souza da Silva, N., da Silva Siqueira, E.M., Tomaz, J.C., Lopes, N.P., Silva-Júnior, A.A., Zucolotto, S.M., Fernandes-Pedrosa, M.F., 2016. Aqueous extract from *Ipomoea asarifolia* (Convolvulaceae) leaves and its phenolic compounds have anti-inflammatory activity in murine models of edema, peritonitis and air-pouch inflammation. *J. Ethnopharmacol.* 192, 225–235.

Gandhi, S., Abramov, A.Y., 2012. Mechanism of oxidative stress in neurodegeneration. *Oxid. Med. Cell. Longev.* 2012, 1–11.

Ganesan, K., Xu, B., 2017. A critical review on polyphenols and health benefits of black soybeans. *Nutr. J.*9, 455.

García-Becerra, L., Mitjans, M., Rivas-Morales, C., Verde-Star, J., Oranday-Cárdenas, A., Vinardell María, P., 2016. Antioxidant comparative effects of two grape pomace Mexican extracts from vineyards on erythrocytes. *Food Chem.* 194, 1081–1088.

Germolec, D.R., Shipkowski, K.A., Frawley, R.P., Evans, E., 2018. Markers of inflammation, in: DeWitt, J.C., Rockwell, C.E., Bowman, C.C. (Eds.), immunotoxicity testing, methods in molecular biology. Springer New York, New York, NY, pp. 57–79.

Ghedadba, N., Hambaba, L., Ayachi, A., Aberkane, M.C., Bousselsela, H., Oueld-Mokhtar, S.M., 2015. Polyphénols totaux, activités antioxydante et antimicrobienne des extraits des feuilles de Marrubium deserti de Noé. *Phytothérapie* 13, 118–129.

Gierlikowska, B., Stachura, A., Gierlikowski, W., Demkow, U., 2021. Phagocytosis, degranulation and extracellular traps release by neutrophils-the current knowledge, pharmacological modulation and future prospects.*Front. Pharmacol* 12.

Gillis, C.M., Reber, L.L., 2018. Un nouveau modèle de souris pour comprendre le rôle des neutrophiles. *médecine/sciences* 34, 339–343.

Ginting, C.N., Lister, I.N.E., Girsang, E., Riastawati, D., Kusuma, H.S.W., Widowati, W., 2020. Antioxidant activities of *Ficus elastica* leaves ethanol extract and its compounds. *Mol. Cell. Biomed. Sci*. 4, 27.

Girard, A., Madani, S., Boukortt, F., Cherkaoui-Malki, M., Belleville, J., Prost, J., 2006. Fructose-enriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats.*Nutrition* 22: 758-766.

Gonçalves, A.C., Bento, C., Silva, B.M., Silva, L.R., 2017. Sweet cherries from Fundão possess antidiabetic potential and protect human erythrocytes against oxidative damage. *Food Res. Int.* 95, 91–100.

Grochot-Przeczek, A., Dulak, J., Jozkowicz, A., 2012. Haem oxygenase-1: non-canonical roles in physiology and pathology. *Clin. Sci.* 122, 93–103.

Grzesik, M., Naparło, K., Bartosz, G., Sadowska-Bartosz, I., 2018. Antioxidant properties of catechins: Comparison with other antioxidants. *Food Chem*. 241, 480–492.

Gülçin, İ., 2012. Antioxidant activity of food constituents: an overview. Arch. Toxicol. 86, 345–391.

Gülçin, İ., Bursal, E., Şehitoğlu, M.H., Bilsel, M., Gören, A.C., 2010. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food Chem. Toxicol.* 48, 2227–2238.

Gupta, K.K., Khan, Mohd, A., Singh, S.K., 2020. Constitutive inflammatory cytokine storm: a major threat to human health. *J. Interferon Cytokine Res.* 40, 19–23.

He, F., Gao, F., Cai, N., Jiang, M., Wu, C., 2022. Chlorogenic acid enhances alveolar macrophages phagocytosis in acute respiratory distress syndrome by activating G protein-coupled receptor 37 (GPR 37). *Phytomedicine* 107, 154474.

Henzelyová, J., Čellárová, E., 2018. Modulation of naphthodianthrone biosynthesis in hairy root derived Hypericum tomentosum regenerants. *Acta Physiol. Plant.*82.

Hosni, K., Msaâda, K., Ben Taârit, M., Ouchikh, O., Kallel, M., Marzouk, B., 2008. Essential oil composition of *Hypericum perfoliatum L*. and *Hypericum tomentosum L.* growing wild in Tunisia. *Ind. Crops Prod.* 27, 308–314.

Jagoda, S.V., Dixon, K.M., 2020. Protective effects of 1,25 dihydroxyvitamin D $_3$ and its analogs on ultraviolet radiation-induced oxidative stress: a review. *Redox Rep.* 25, 11–16.

Jakimiuk, K., Gesek, J., Atanasov, A.G., Tomczyk, M., 2021. Flavonoids as inhibitors of human neutrophil elastase. *J. Enzyme Inhib. Med. Chem.* 36, 1016–1028.

Jayasena, T., Poljak, A., Smythe, G., Braidy, N., Münch, G., Sachdev, P., 2013. The role of polyphenols in the modulation of sirtuins and other pathways involved in Alzheimer's disease. *Ageing Res. Rev.* 12, 867–883.

Joffre, C., Layé, S., 2016. Nutrition, inflammation et dépression. *Corresp. en Métabolismes Horm. Diabètes Nutr* 20.

Jomova, K., Lawson, M., Drostinova, L., Lauro, P., Poprac, P., Brezova, V., Michalik, M., Lukes, V., Valko, M., 2017. Protective role of quercetin against copper (II)-induced oxidative stress: A spectroscopic, theoretical and DNA damage study. *Food Chem. Toxicol.* 110, 340–350.

Jones, W.P., Kinghorn, A.D. (Eds.), 2005. Extraction of plant secondary metabolites, in: natural products isolation, methods in biotechnology. Humana Press, Totowa, N.J
.

Jovanović, A.A., Đorđević, V.B., Zdunić, G.M., Pljevljakušić, D.S., Šavikin, K.P., Gođevac, D.M., Bugarski, B.M., 2017. Optimization of the extraction process of polyphenols from *Thymus serpyllum L.* herb using maceration, heat- and ultrasound-assisted techniques. *Sep. Purif. Technol.*

Jung, M., Dodsworth, M., Thum, T., 2019. Inflammatory cells and their non-coding RNAs as targets for treating myocardial infarction. *Basic Res. Cardiol*. 114, 4.

Ka, M.M., Diop, M.M., Touré, P.S., Berthe, A., Diop, B.M., 2019. Les enjeux de la biothérapie en Afrique. *Rev. Afr. Médecine Interne* 6.

Kamal, K., AbdoAlwahab, A., Esmail, A.E., 2019. Glutathione and selenium as chemopreventive agents on experimentally induced hamster buccal pouch epithelial dysplasia. *Egypt. Dent. J.* 65, 331–342.

Kato, Y., 2016. Neutrophil myeloperoxidase and its substrates: formation of specific markers and reactive compounds during inflammation.*J. Clin. Biochem. Nutr.* 58, 99–104.

Kernouf, N., Bouriche, H., Kada, S., Messaoudi, D., Assaf, A., Senator, A., 2018. Anti-inflammatory and immuno-modulatory effects of *Capparis spinosa* flower bud extract. *Annu. Res. Rev. Biol.* 25, 1–11.

Khan, A., Muhamad, N.A., Ismail, H., Nasir, A., Khalil, A.A.K., Anwar, Y., Khan, Z., Ali, A., Taha, R.M., Al-Shara, B., Latif, S., Mirza, B., Fadladdin, Y.A.J., Zeid, I.M.A., Al-Thobaiti, S.A., 2020. Potential nutraceutical benefits of *in vivo* grown saffron (*Crocus sativus L.*) as analgesic, anti-inflammatory, anticoagulant, and antidepressant in mice. *Plants* 9, 1414.

Khokhar, S., Owusu Apenten, Richard, K., 2003. Iron binding characteristics of phenolic compounds: some tentative structure–activity relations. *Food Chem.* 81, 133–140.

Kim, I.-S., Yang, M.-R., Lee, O.-H., Kang, S.-N., 2011. Antioxidant activities of hot water extracts from various spices. *Int. J. Mol. Sci.* 12, 4120–4131.

Kim, M.-H., 2016. Biological effects of zinc oxide nanoparticles on inflammation. *Tang Humanit. Med.* 6, 23.1-23.6.

Kourtzelis, I., Mitroulis, I., Renesse, J., Hajishengallis, G., Chavakis, T., 2017. From leukocyte recruitment to resolution of inflammation: the cardinal role of integrins. *J. Leukoc. Biol.* 102, 677–683.

Kumar, T., Jain, V., 2014. Antinociceptive and anti-inflammatory activities of *Bridelia retusa* methanolic fruit extract in experimental animals. *Sci. World J.* 2014, 1–12.

Kwon, K.-R., Alam, M.B., Park, J.-H., Kim, T.-H., Lee, S.-H., 2019. Attenuation of UVB-induced photo-aging by polyphenolic-rich *Spatholobus suberectus* stem extract via modulation of MAPK/AP-1/MMPs signaling in human keratinocytes. *Nutr.J.* 11.

Muthu, T.L., Radha, R., Jayshree, N., 2014. In vitro antioxidant activity, total phenolic and total flavonoid content IN extracts from the bark of dalbergia sissoo roxb. *Int J Pharm Sci Res*5 (5), 226-231.

Li, H.B., Cheng,K.W., Wong,C.C., Fan, K.W., Chen, F., Jiang, Y., 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chem*, 102, 771-776.

Li, P., Stetler, R.A., Leak, R.K., Shi, Y., Li, Y., Yu, W., Bennett, M.V.L., Chen, J., 2018. Oxidative stress and DNA damage after cerebral ischemia: Potential therapeutic targets to repair the genome and improve stroke recovery.*Neuropharmacology* 134, 208–217.

Li, X., Jiang, Q., Wang, T., Liu, J., Chen, D., 2016. Comparison of the antioxidant effects of quercitrin and isoquercitrin: understanding the role of the 6″-OH group. *ISO4* 21.

Liang, N., Sang, Y., Liu, W., Yu, W., Wang, X., 2018. Anti-inflammatory effects of gingerol on lipopolysaccharide-stimulated raw 264.7 cells by inhibiting NF-κB signaling pathway. *Inflammation* 41, 835–845.

Liberman, A.C., Budziñski, M.L., Sokn, C., Gobbini, R.P., Steininger, A., Arzt, E., 2018. Regulatory and mechanistic actions of glucocorticoids on T and inflammatory cells. *Front. Endocrinol.* 9, 235.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 13, 757–772.

Ling, L., Lu, Y., Zhang, Y., Zhu, H., Tu, P., Li, H., Chen, D., 2020. Flavonoids from *Houttuynia cordata* attenuate H1N1-induced acute lung injury in mice via inhibition of influenza virus and Toll-like receptor signalling. *Phytomedicine* 67.

Lopatko Fagerström, I., Ståhl, A., Mossberg, M., Tati, R., Kristoffersson, A.-C., Kahn, R., Bascands, J.-L., Klein, J., Schanstra, J.P., Segelmark, M., Karpman, D., 2019. Blockade of the kallikrein-kinin system reduces endothelial complement activation in vascular inflammation. *BioMedicine* 47, 319–328.

Lopes Neto, J.J., Silva de Almeida, T., Almeida Filho, L.C.P., Rocha, T.M., Nogara, P.A., Nogara, K.F., Teixeira da Rocha, J.B., Almeida Moreira Leal, L.K., Urano Carvalho, A.F., 2020. Triplaris gardneriana seeds extract exhibits in vitro anti-inflammatory properties in human neutrophils after oxidative treatment. *J. Ethnopharmacol.* 250.

Makarova, K., Sajkowska-Kozielewicz, J.J., Zawada, K., Olchowik-Grabarek, E., Ciach, M.A., Gogolewski, K., Dobros, N., Ciechowicz, P., Freichels, H., Gambin, A., 2021. Harvest time affects antioxidant capacity, total polyphenol and flavonoid content of Polish St John's wort's (Hypericum perforatum L.) flowers. *Sci. Rep.* 11.

Manga, H.M., Brkic D., Marie, D.E.P., Quetin-Leclercq, J., 2004. *In vivo* anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae). *J Ethnopharmacol*, 92, 209-214.

Marinaccio, L., Stefanucci, A., Scioli, G., Della Valle, A., Zengin, G., Cichelli, A., Mollica, A., 2022. Peptide human neutrophil elastase inhibitors from natural sources: an overview. *Int. J. Mol. Sci.* 23, 2924.

Mártonfiová, L., Danova, K., Toteva, V.K., 2014. Karyotype analysis of *Hypericum rumeliacum* boiss. *Thaiszia - J. Bot., Košice,* 24, 143–150.

Maruhashi, T., Kihara, Y., Higashi, Y., 2019. Bilirubin and endothelial function. *J. Atheroscler. Thromb.* 26, 688–696.

Mederos-Molina, S., 2002. Micropropagation of *Hypericum canariense L*. for the production of hypericin, in: Nagata, T., Ebizuka, Y. (Eds.), medicinal and aromatic plants XII, biotechnology in agriculture and forestry. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 95–117.

Mescher, A.L., 2017. Macrophages and fibroblasts during inflammation and tissue repair in models of organ regeneration. *Regeneration* 4, 39–53.

Miara, M.D., Bendif, H., Rebbas, K., Rabah, B., Hammou, M.A., Maggi, F., 2019. Medicinal plants and their traditional uses in the highland region of Bordj Bou Arreridj (Northeast Algeria). *J. Herb. Med.* 16.

Migdal, C., Serres, M., 2011. Espèces réactives de l'oxygène et stress oxydant. *médecine/sciences* 27, 405–412.

Mohammedi, H., Idjeri-Mecherara, S., Menaceur, F., Azine, K., Hassani, A., 2018. Chemical compositions of extracted volatile oils of *Ammodaucus leucotrichus* L. Fruit from different geographical regions of Algeria with evaluation of its toxicity, anti-inflammatory and antimicrobial activities. *J. Essent. Oil Bear. Plants* 21, 1568–1584.

Monteiro, B., Steagall, P.V., 2019. Antiinflammatory drugs. *Vet. Clin. North Am. Small Anim. Pract.* 49, 993–1011.

Müller, H.E., 1985. Detection of hydrogen peroxide produced by microorganisms on an ABTS peroxidase medium. Zentralbl. Bakteriol. *MikroBiol. Hyg. Med. Microbiol. Infect. Dis. Virol. Paras.* 259 (2), 151–154.

Napoli, E., Siracusa, L., Ruberto, G., Carrubba, A., Lazzara, S., Speciale, A., Cimino, F., Saija, A., Cristani, M., 2018. Phytochemical profiles, phototoxic and antioxidant properties of eleven Hypericum species – A comparative study. *Phytochemistry* 152, 162–173.

Nasimuzzaman, M., Malik, P., 2019. Role of the coagulation system in the pathogenesis of sickle cell disease. *Blood Adv.* 3, 3170–3180.

Nemmiche, S., 2016. Oxidative signaling response to cadmium exposure. *Toxicol. Sci.* kfw222.

Ng, Z.X., Samsuri, S.N., Yong, P.H., 2020. The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. J. Food Process. *Preserv.* 44.

Noack, M., Kolopp-Sarda, M.-N., 2018. Cytokines et inflammation : physiologie, physiopathologie et utilisation thérapeutique. *Rev. Francoph. Lab.* 2018, 28–37.

Nokhbehsaim, M., Nogueira, A.V.B., Nietzsche, S., Eick, S., Deschner, J., 2020. Regulation of cyclooxygenase 2 by *Filifactor alocis* in fibroblastic and monocytic cells. *Mediators Inflamm.* 2020, 1–8.

OECD, 2001. OECD guideline for testing of chemicals. Acute oral toxic. – fixed dose Proced. 420 Adopted: 17th December 2001.

Olivares-Vicente, M., Barrajon-Catalan, E., Herranz-Lopez, M., Segura-Carretero, A., Joven, J., Encinar, J.A., Micol, V., 2018. Plant-derived polyphenols in human health: biological activity, metabolites and putative molecular targets. *Curr. Drug Metab.* 19, 351–369.

Ortega, E., Collazos, M.E., Maynar, M., Barriga, C., De la Fuente, M., 1993. Stimulation of the phagocytic function of neutrophils in sedentary men after acute moderate exercise. *Eur. J. Appl. Physiol.* 66, 60–64.

Park, M.H., Kim, D.H., Lee, E.K., Kim, N.D., Im, D.S., Lee, J., Yu, B.P., Chung, H.Y., 2014. Age-related inflammation and insulin resistance: a review of their intricate interdependency. *Arch. Pharm. Res.* 37, 1507–1514.

Parker, H.A., Dickerhof, N., Forrester, L., Ryburn, H., Smyth, L., Messens, J., Aung, H.L., Cook, G.M., Kettle, A.J., Hampton, M.B., 2021. *Mycobacterium smegmatis* resists the bactericidal activity of hypochlorous acid produced in neutrophil phagosomes. *J. Immunol.* 206, 1901–1912.

Patil, K.R., Patil, C.R., 2017. Anti-inflammatory activity of bartogenic acid containing fraction of fruits of *Barringtonia racemosa* Roxb. in acute and chronic animal models of inflammation. *J. Tradit. Complement. Med.* 7, 86–93.

Peng, C., Wang, X., Chen, J., Jiao, R., Wang, L., Li, Y.M., Zuo, Y., Liu, Y., Lei, L., Ma, K.Y., Huang, Y., Chen, Z.-Y., 2014. Biology of ageing and role of dietary antioxidants. *BioMed Res. Int.* 2014, 1–13.

Picard, F., Adjedj, J., Varenne, O., 2017. Le diabète, une pathologie prothrombotique. *Ann. Cardiol. Angéiologie* 66, 385–392.

Piwowarski, J.P., Kiss, A.K., Kozłowska-Wojciechowska, M., 2011. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background. *J. Ethnopharmacol.* 137, 937–941.

Popova, A., 2020. Oxidative stress and plant deriving antioxidants. *Asian J. Green Chem.* 4, 121–133.

Prahadeesh, N., Sithambaresan, M., Mathiventhan, U., 2018. A Study on hydrogen peroxide scavenging activity and ferric reducing ability of simple coumarins. *Emerg. Sci. J.* 2, 417.

Prokopowicz, Z., Marcinkiewicz, J., Katz, D.R., Chain, B.M., 2012. Neutrophil Myeloperoxidase: Soldier and Statesman. *Arch.Immunol. Ther. Exp.* 60, 43-54.

Ramalhete, N., Machado, A., Serrano, R., Gomes, E.T., Mota-Filipe, H., Silva, O., 2016. Comparative study on the in vivo antidepressant activities of the Portuguese *Hypericum foliosum*, *Hypericum androsaemum* and *Hypericum perforatum* medicinal plants. *Ind. Crops Prod.* 82, 29–36.

Rebaya, A., Belghith, S.I., 2016. Total phenolic compounds and antioxidant potential of rokrose (*Cistus salviifolius*) leaves and flowers grown in Tunisia. *Int. J. Pharmacogn. Phytochem. Res.*8, 327-331

Ren, J., Liao, L., Shang, S., Zheng, Y., Sha, W., Yuan, E., 2019. Purification, characterization, and bioactivities of polyphenols from *Platycladus orientalis* (L.) FRANCO: PF from platycladus orientalis Franco leaves. *J. Food Sci.* 84, 667–677.

Rostan, O., Tarte, K., Amé-Thomas, P., 2014. Le polynucléaire basophile: nouveautés en physiopathologie et implications diagnostiques. *Rev. Francoph. Lab.* 2014, 95–105.

Rouis, Z., Laamari, A., Abid, N., Elaissi, A., Cioni, P.L., Flamini, G., Aouni, M., 2012. Chemical composition and larvicidal activity of several essential oils from *hypericum* species from Tunisien.*Parasitol. Res*.

Rousselet, M., Vignaud, J.M., 2005. Inflammation et pathologie inflammatoire (Chapitre 3). *Copyr. AFECAP*.

Saddhe, A.A., Malvankar, M.R., Karle, S.B., Kumar, K., 2019. Reactive nitrogen species: Paradigms of cellular signaling and regulation of salt stress in plants. *Environ. Exp. Bot.* 161, 86–97.

Sánchez-Mateo, C.C., Bonkanka, C.X., Hernández-Pérez, M., Rabanal, R.M., 2006. Evaluation of the analgesic and topical anti-inflammatory effects of *Hypericum reflexum L*. fil. J. Ethnopharmacol. 107, 1–6.

Sansbury, B.E., Spite, M., 2016. Resolution of acute inflammation and the role of resolvins in immunity, thrombosis, and vascular biology. *Circ. Res.* 119, 113–130.

Santos, C.C. de M.P., Salvadori, M.S., Mota, V.G., Costa, L.M., de Almeida, A.A.C., de Oliveira, G.A.L., Costa, J.P., de Sousa, D.P., de Freitas, R.M., de Almeida, R.N., 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. *Neurosci. J.* 2013, 1–9.

Santos, I.B. da S., Santos dos Santos, B., Oliveira, J.R.S. de, Costa, W.K., Zagmignan, A., da Silva, L.C.N., Ferreira, M.R.A., Lermen, V.L., Lermen, M.S.B. de S., da Silva, A.G., Ximenes, R.M., Soares, L.A.L., Paiva, P.M.G., Lima, V.L. de M., Correia, M.T. dos S., da Silva, M.V., 2020. Antioxidant action and *in vivo* anti-inflammatory and antinociceptive activities of *Myrciaria floribunda* fruit peels: possible involvement of opioidergic system. *Adv. Pharmacol. Pharm. Sci.* 2020, 1–11.

Šavikin, K., Zucolotto, S.M., TadiÇ, V., ZduniÇ, G., 2006. Antiinflammatory activity of ethanol extracts of *Hypericum perforatum* L., *H. barbatum* Jacq., *H. hirsutum* L., *H. richeri Vill*. and *H. androsaemum* L. in rats. *Phytother. Res.* 21, 176–180.

Schepetkin, I., Özek, G., Özek, T., Kirpotina, L., Khlebnikov, A., Quinn, M., 2020. Chemical composition and immunomodulatory activity of *Hypericum perforatum* essential oils. *Biomolecules* 10.

Schmidt, H.M., Kelley, E.E., Straub, A.C., 2019. The impact of xanthine oxidase (XO) on hemolytic diseases. *Redox Biol.* 21.

Seidel, V. (Ed.), 2012. Initial and bulk extraction of natural products isolation, in: Natural products isolation, methods in molecular biology. Humana Press, Totowa, NJ.

Seidel, V., 2006. Initial and bulk extraction, in: natural products isolation. Humana Press (Totowa), New Jersey.

Şerbetçi, T., Özsoy, N., Demirci, B., Can, A., Kültür, Ş., Başer, K.H.C., 2012. Chemical composition of the essential oil and antioxidant activity of methanolic extracts from fruits and flowers of *Hypericum lydium* Boiss. *Ind. Crops Prod.* 36, 599–606.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. *J. Funct. Foods* 18, 820–897.

Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S., Mardani, F., Seifi, B., Mohammadi, A., Afshari, J.T., Sahebkar, A., 2018. Macrophage plasticity, polarization, and function in health and disease. *J. Cell. Physiol.* 233, 6425-6440.

Sharma, P., Ravikumar, G., Kalaiselvi, M., Gomathi, D., Uma, C., 2013. *In vitro* antibacterial and free radical scavenging activity of green hull of *Juglans regia*. *J. Pharm. Anal.* 3 (4), 298–302.

Song, P., Zou, M.-H., 2015. Roles of reactive oxygen species in physiology and pathology, in: Wang, H., Patterson, C. (Eds.), Atherosclerosis. John Wiley & Sons, Inc, Hoboken, NJ, pp. 379–392.

Sosa, S., Pace, R., Bornanciny, A., Morazzoni, P., Riva, A., Tubaro, A., Loggia, R.D., 2006. Topical anti-inflammatory activity of extracts and compounds from *Hypericum perforatum* L. *J. Pharm. Pharmacol.* 59, 703–709.

Strehl, C., Spies, C.M., Buttgereit, F., 2011. Pharmacodynamics of glucocorticoids. *Pharmacodyn. Glucocorticoids*.13-18

Svoboda, M., Fajt, Z., Vašek, J., Vaňhara, J., Hostovský, M., Blahová, J., Franc, A., 2016. Effect of parenteral selenium administration on oxidative status of weaned piglets. *Acta Vet. Brno* 85, 377–386.

Tarasiuk, A., Bulak, K., Talar, M., Fichna, J., 2021. Chlorogenic acid reduces inflammation in murine model of acute pancreatitis. *Pharmacol. Rep.* 73, 1448–1456.

Taverna, M., Marie, A.-L., Mira, J.-P., Guidet, B., 2013. Specific antioxidant properties of human serum albumin. *Ann. Intensive Care* 3.

Torres-Rêgo, M., Furtado, A.A., Bitencourt, M.A.O., Lima, M.C.J. de S., Andrade, R.C.L.C. de, Azevedo, E.P. de, Soares, T. da C., Tomaz, J.C., Lopes, N.P., da Silva-Júnior, A.A., Zucolotto, S.M., Fernandes-Pedrosa, M. de F., 2016. Anti-inflammatory activity of aqueous extract and bioactive compounds identified from the fruits of Hancornia speciosa Gomes (Apocynaceae). BMC Complement. *Altern. Med*. 16, 275.

Touafek, O., 2010. Etude phytochimique de plantes medicinales du nord et du sud Algeriens, thesis of doctorat. Constantine, Algeria.

Touafek, O., Kabouche, Z., Boustie, J., Bruneau, C., 2012a. A new long-chain unsaturated ester and other constituents of *Hypericum tomentosum*. *Nat. Prod. Commun.*7

Touafek, O., Mesbah, L., Mekhloufi, E., Benguedour, L., Boustie, J., Bruneau, C., Kabouche, Z., 2012b. Compents and antioxidant activity of Hypericum tomentosum L. (clusiaceae). *JBAPN*. 2(3), 158-166

Tsumbu, C.N., Deby-Dupont, G., Tits, M., Angenot, L., Frederich, M., Kohnen, S., Mouithys-Mickalad, A., Serteyn, D., Franck, T., 2012. Polyphenol content and modulatory activities of some tropical dietary plant extracts on the oxidant activities of neutrophils and myeloperoxidase. *Int. J. Mol. Sci.* 13, 628–650.

Valentão, P., Fernandes, E., Carvalho, F., Andrade, P.B., Seabra, R.M., Bastos, M. de L., 2002. Antioxidant activity of *Hypericum androsaemum* infusion: Scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. *Biol. Pharm. Bull.* 25, 1320–1323.

Varela, M.L., Mogildea, M., Moreno, I., Lopes, A., 2018. Acute inflammation and metabolism. *Inflammation* 41, 1115–1127.

Veith, A., Moorthy, B., 2018. Role of cytochrome P450s in the generation and metabolism of reactive oxygen species. *Curr. Opin. Toxicol.* 7, 44–51.

Vezzani, A., Friedman, A., Dingledine, R.J., 2013. The role of inflammation in epileptogenesis. *Neuropharmacology* 69, 16–24.

Vidhya, R., Anbumani, V.I., Dinakara Rao, A., Anuradha, C.V., 2022. Identification of novel human neutrophil elastase inhibitors from dietary phytochemicals using *in silico* and *in vitro* studies. *J. Biomol. Struct. Dyn.* 40, 3451–3461.

Voiriot, G., Chalumeau, M., Messika, J., Basille, D., Philippe, B., Ricard, J.-D., Andrejak, C., Jounieaux, V., Sanchez, O., Fartoukh, M., 2018. Risques associés à la prise d'anti-inflammatoires non stéroïdiens au cours de la pneumonie. *Rev. Mal. Respi*r. 35, 430-440.

Wang, Z., Yi, K., Lin, Q., Yang, L., Chen, X., Chen, H., Liu, Y., Wei, D., 2019. Free radical sensors based on inner-cutting graphene field-effect transistors.*Nat. Commun*. 10, 1544.

Wanikiat, P., Panthong, A., Sujayanon, P., Yoosook, C., Rossi, A.G., Reutrakul, V., 2008. The anti-inflammatory effects and the inhibition of neutrophil responsiveness by Barleria lupulina and Clinacanthus nutans extracts. *J. Ethnopharmacol.* 116, 234–244.

Wong, S., Leong, L., Williamkoh, J., 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.* 99, 775–783.

Xiao, F., Xu, T., Lu, B., Liu, R., 2020. Guidelines for antioxidant assays for food components. *Food Front.* 1, 60–69.

Xu, Y.-Q., Gao, Y., Granato, D., 2021. Effects of epigallocatechin gallate, epigallocatechin and epicatechin gallate on the chemical and cell-based antioxidant activity, sensory properties, and cytotoxicity of a catechin-free model beverage. *Food Chem.* 339.

Yaribeygi, H., Atkin, S.L., Sahebkar, A., 2019. A review of the molecular mechanisms of hyperglycemia-induced free radical generation leading to oxidative stress. *J. Cell. Physiol.* 234, 1300–1312.

Yilmaz, M.A., 2020. Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC–MS/MS method validation. *Ind. Crops Prod.* 149, 112347.

Yonathan, M., Asres, K., Assefa, A., Bucar, F., 2006. In vivo anti-inflammatory and anti-nociceptive activities of Cheilanthes farinosa.*J. Ethnopharmacol.* 108, 462–470.

Zhang, H., Tsao, R., 2016. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Curr. Opin. Food Sci.* 8, 33–42.

Zhang, Q.-W., Lin, L.-G., Ye, W.-C., 2018. Techniques for extraction and isolation of natural products: a comprehensive review.*Chin. Med.* 13, 20.

Zhang, X., Meng, X., Chen, Y., Leng, S.X., Zhang, H., 2017. The biology of aging and cancer. *Cancer J.* 23.

Zhang, Z., Lv, Z., Shao, Y., Qiu, Q., Zhang, W., Duan, X., Li, Y., Li, C., 2017. Microsomal glutathione transferase 1 attenuated ROS-induced lipid peroxidation in Apostichopus japonicus. *Dev. Comp. Immunol.* 73, 79–87.

Zhao, Y., Lim, J., Xu, J., Yu, J., Zheng, W., 2020. Nitric oxide as a developmental and metabolic signal in filamentous fungi. *Mol. Microbiol.* 113, 872–882.

Zobayed, S.M.A., Afreen, F., Goto, E., Kozai, T., 2006. Plant–Environment Interactions: Accumulation of Hypericin in Dark Glands of *Hypericum perforatum*. *Ann. Bot.* 98, 793–804.

Zubrická, D., Mišianiková, A., Henzelyová, J., Valletta, A., De Angelis, G., D'Auria, F.D., Simonetti, G., Pasqua, G., Čellárová, E., 2015. Xanthones from roots, hairy roots and cell suspension cultures of selected *Hypericum* species and their antifungal activity against *Candida albicans*. *Plant Cell Rep.* 34, 1953–1962

Zulaikhah, S.T., 2017. The role of antioxidant to prevent free radicals in the body. *SainsMed* 8

PUBLICATION

Indian Journal of Novel Drug Delivery 14(4), Oct-Dec, 2022, 192-199

Indian Journal of Novel Drug Delivery IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Antioxidant activity of aqueous extract of leaves from *Hypericum tomentosum*

LAMOURI AMINA*, MESSAOUDI DALILA, KADA SEOUSSEN, BOURICHE HAMAMA, SENATOR ABDERRAHMANE

Laboratory of Applied Biochemistry, Faculty of Natural Science and Life, University of Ferhat Abbas, Sétif 1, Sétif 19000, Algeria

ARTICLE DETAILS

Article history:
Received on 9 September 2022
Modified on 16 November 2022
Accepted on 20 November 2022

Keywords:
Antioxidant,
Hypericum tomentosum,
Oxidative Stress,
Polyophenols, Flavonoids.

ABSTRACT

Hypericum tomentosum (*H. tomentosum*) is a medicinal plant distributed in Mediterranean region, it's commonly used in folk medicine for different health problems. Thus, this study aimed to investigate the antioxidant properties of aqueous extract of leaves of *H. tomentosum*. The aqueous extract was obtained by boiling *H. tomentosum* leaves in distilled water. The total phenolics and flavonoids content were determined using folin-ciocalteu's reagent and aluminum chloride, respectively. The aqueous extract of *H.tomentosum* was tested for antioxidant activity as determined by DPPH• radical and hydrogen peroxide scavenging assays, reducing power assay, ferrous ion chelating and the ability of this extract to prevent hemolysis were also performed. The result showed that aqueous extract of *H.tomentosum possess* high levels of polyphenols (303.10 ± 5.14 µg GAE/mg extract) and flavonoids (53.56 ± 1.74 µg QE/ mg extract). *H .tomentosum* exerted a powerful scavenging activity against DPPH• radical and hydrogen peroxide with EC_{50} values of 9.32 ± 0.96 µg/mL and 3.13 ± 0.49 µg/mL, respectively. Moreover, it exerted a powerful reducing ability and inhibits ferrous ion chelating .The extract exhibited a significant protective effect against AAPH-induced erythrocyteshemolysis. This study suggests that *H.tomentosum* may represent a prospective source of natural antioxidants to prevent and /or to treat oxidative stress related diseases.

INTRODUCTION

An imbalance in the oxidant/antioxidant system, either resulting from excessive reactive oxygen species (ROS) production and/or antioxidant system impairment, leads to oxidative stress that cause damage to cells and tissue [1,2]. Thereactive oxygen species include radical species such as superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$), and non-radical species such as hydrogen peroxide (H_2O_2) [3]. ROS may be generated by ultraviolet rayon [4], xenobiotic as alcohol, medicament and tobacco; transited metal as plumb and cobalt may induce theformation of this species [5]. ROS may be generated also by intracellular sources. In living organism, electron-transport chain mitochondrial is the main part of ROS production. Other enzyme like xanthine oxidase and NADPH oxidase participate in their formation [6–8].

The excess of uncontrolled ROS production leads to cell damage and homeostatic disruption. They can react with polyunsaturated fatty acids of cell membranes and induce lipid peroxidation causing the generation of secondary free radicals [6, 9]. They can also attack protein and DNA [10, 11] bringing about different pathologies such ascancer, cataract, edema pulmonary, accelerated aging, diabetes and cardiovascular diseases [12, 13]. Synthetic antioxidants play an important role to protect human body against damage caused by ROS; however, they cause many side effects [14]. Plants have been used for traditional medicine throughout the world since ancient times. Mostof 80% countries in the world used medicinalplants for healthy purposes [15]. In recent years, interest has increased in naturally bioactive compounds that can preserve human health from oxidative stress damage, and inhibit enzymes involved in several diseases [16].

Hypericum tomentosum (Clusiaceae) is a medicinal plant which is mainly distributed in

Author for Correspondence:
Email: lamouri.amina93@gmail.com

Mediterranean region [17]. Its distribution areas were restricted to the humid forest [18]. *H. tomentosum* is used by traditional healers for its anti-inflammatory, antioxidant, antiviral and analgesic effects [19], it is employed also for treatment of depression, ulcer, rheumatism and hysteria [20]. According to Naboli et al. [21] *Hypericum* species have many important chemical compounds possessing biological activities. Therefore, the current study was designed to evaluate the antioxidant potency of *H.tomentosum* aqueous extracts against oxidativedamages.

Materials and methods

1. Chemicals

2,2′ -azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferrous sulfate ($FeSO_4$), peroxidase, folin-ciocalteu'sreagent,2,2'-diphenyl-1- picrylhydrazyl (DPPH•),quercetin, gallic acid, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), ferric chloride (III) ($FeCl_3$), butylated hydroxytoluene (BHT), EDTA, ferrous chloride ($FeCl_2$), ferrozine and Trolox were obtained from Sigma-Aldrich (Darmstadt, Germany). Aluminium chloride ($AlCl_3$) and sodium carbonate (Na_2CO_3) were purchased from Prolabo (Paris, France). Sodium dihydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4) and Sodium chloride (NaCl) were acquired from Panreac (Panreac, Spain). Hydrogen peroxide (H_2O_2) and methanol of the greatest available chemical purity were provided from Riedel-de Haen (Illkirch, France).

2. Plants Material

Hypericum tomentosum was collected in June 13th, 2020 from Serdj elghoul, Setif in Algeria. The plant was identified by Pr. H. Laouer, sétif 1 university, Algeria. The aerial parts were dried in room temperature and in shadow. Then Air- dried leaves were ground using an electric grinder (sayona szj-1306, china) in order to get a fine powder.

3. Animals

Female *Swiss Albinos* mice were obtained from Pasteur Institute of Algeria. Procedure using animals were performed in accordance with European Union Guidelines for Animals Experimentation (2007/526/EC).

4. Preparation of Extraction

Aqueous extract was prepared according to Gülçin et al. [22]. 50 g of powdered dried leaves were boiling in 800 mL of distilled water for 15 min (at solid-liquid ration of 1:16). After cooling the mixture was filtrated and the extract was lyophilized to obtain a red fine powered which was stored at -32°C until use.

5. Phytochemical Analysis

5. 1. Determination of Total Phenolic Content

Total phenolic content of aqueous was determinate as describe by Messaoudi et al. [23]. Practically, 100 µL of extract were mixed with500 µl of folin-ciocalteu's reagent (10%). After 4 minutes, 400 µl of Na_2CO_3 (7.5%) was added and the reaction mixture was incubated in obscurity at ambient temperature for 2 hours followed by the measuring of absorbance at 765 nm usingUV-VS spectrophotometer (Thermo Spectronic,USA). Gallic acid was used as standard for thecalibration curve. Results were expressed as µgGallic Equivalents per mg of extract (µg GAE/ mgof extract). The sample was analyzed three timesand the mean value was calculated.

5. 2. Determination of Total Flavonoid Content

Flavonoid content was evaluated usingcolorimetric method according to Kada et al. [24]. An aliquot of 0.5 mL of sample was mixed with the same volume of $AlCl_3$ (2% prepared in methanol). After incubation in obscurity for 10 min at ambient temperature, the absorbance wasmeasured at 430 nm. The standard curve was established using quercetin as standard and the results were expressed as µg Quercetin Equivalent (QE) per mg of extract (µg QE/ mg of extract).

6. Antioxidant Activities

6. 1. DPPH• Radical Scavenging Activity

The ability of extract to scavenge DPPH• radical was evaluated as described by Que et al. [25]. Practically 0.5 mL of DPPH• (0.1 mM dissolved in methanol) was mixed with 0.5 mL of differentconcentrations of *H.tomentosum* aqueous extract (2- 65 µg/mL). The mixture was incubated in obscurity for 30 minutes. The absorbance wasmeasured at 517 nm. Trolox was used as standard and the percentage of radical scavenging activity was calculated using following formula:

DPPH• scavenging activity (%) = ($A_{control} - A_{sample}$) x $100/A_{control}$

Where:
$A_{control}$: absorbance of the control reaction mixture without the test compounds, and,
A_{sample}: absorbance of sample reaction mixture with test compounds.

6. 2. Hydrogen Peroxide Scavenging Activity

The capacity of *H.tomentosum* to trap the non-radical reactive specie hydrogen peroxide was estimated as described by Kherbache et al. [26]. Briefly, in 96 wells microplate, 20 µl of extract at different concentrations was incubated with 100 µL of sodium phosphate buffer solution (PBS)(0.1 M, pH 5) and H_2O_2 (20 µL; 10 mM) for 5min at 37°C, followed by the addition of 30 µl ABTS (1.25 mM) and 30 µl of peroxidase (1 U/mL)then incubated again for 15 min at 37°C. The absorbance was read at λ = 405 nm using a microplate reader (ELX 800, Bio-TEK instruments, Winooski, VT, USA), ascorbic acid was used as standard in this test.

6. 3. Reducing Power Activity

Reducing power of aqueous extract of *H. tomentosum* was determined according to Kadaet al. [24]. 200 µl of extract at different concentrations (2.5-50 µg/ mL) was incubatedwith 425 µl of PBS (0.2 M, pH 6.6) and 625 µl of potassium ferricyanide 1% (prepared in PBS) for 20 min at 50°C. After cooling 625µl of TCA (10% in PBS) was added. 625 µl of each mixture was transferred in other tubes that contain 625 µl of distilled water and 125 µl $FeCl_3$ 0.1 % and the absorbance was measured subsequently at 700 nm against blank without extract. BHT (2.5-50 µg/ mL) was used as standard antioxidant.

6. 4. Ferrous Ion Chelating Activity

H. tomentosum chelating ferrous ion was evaluated as describe by Le et al. [27]. Briefly, 700 µL of extract at different concentrations (0.1 – 3 mg/ mL) were added to 50 µl $FeCl_2$ 0.6 mM. After 5 min of incubation, the reaction was initiated by addition of 50 µl of ferrozine 5 mM (prepared in 80% methanol). The reaction was incubation again for 10 min, a necessary time for complex (Fe (II)-Ferrozine) formation with violet color which has a high absorbance at 562 nm. EDTA (1- 30 µg/mL) was used as reference.

The chelating activity of extract and EDTA was calculated according to the equation:

$$\text{Chelating activity}(\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

$A_{control}$: absorbance of the control
A_{sample}: absorbance of sample

6. 5. Anti-Hemolytic Activity

Anti-hemolytic effect of *H. tomentosum* aqueous extract was assessed according to the procedure described by Meziti et al. [28]. Briefly, blood was collected in heparinized syringe through direct heart puncture from anesthetized female *Swiss albinos* mouse and then diluted to 2% in physiological buffer (NaCl 125 mM, sodium phosphate 10 mM, pH 7.4). In a 96 well plate, 120 µl of erythrocytes suspension were pre-incubated with 60 µl of different concentrations of the extract (5, 10, 25 and 50 µg/mL) or Trolox (25 and 50 µg/mL) for 30 minutes at 37°C. To each well, 120 µl of AAPH (120 mM dissolved in cold physiological buffer) were then added and the mixture was incubated at 37°C. The kinetic of hemolysis was monitored for 4 hours by measuring reductions in turbidity at 630 nm, using a 96-well microplate reader (ELX 800, Bio- TEK instruments, Winooski, VT, USA). The physiological buffer served as the control while erythrocytes not treated by AAPH were used as negative control to remove auto hemolysis of erythrocytes.

7. Statistical Analysis

Experiments were performed in triplicate. The results are expressed as mean ± standard error. The significance of differences (P <.001) among treatment means was determined by analysis of variance (one-way ANOVA) using GraphPad Prism 8 software.

RESULTS AND DISCUSSION

1. Phytochemical Analysis

Amount of total phenols and flavonoids in the *H. tomentosum* leaves aqueous extract are reported in Table 1. The content of polyphenols arereported as Gallic Acid Equivalents (GAE) by reference to standard curve (y = 0.0099x -0.0997, R^2= 0.9981). Amount of total flavonoids are reported as Quercetin Equivalents (QE) by reference to standard curve (y = 0.037x -0.0085, R^2 = 0.9993). There are a very few studies in the literature that focused on phenolic content of *H. tomentosum.* Although, almost 500 species of this genus have been documented. But only 40 % of them have been phytochemically characterized [29].

Table1: Total phenols and flavonoids content of aqueous extract of leaves of *H. tomentosum*

Polyphenols		Flavonoids
Amount	303.10 ± 5.14 µg GAE/ mg of extract	53.56 ±1.74 µg QE/mg of extract
Standard curve	y = 0.0099x-0.0997	y = 0.037x-0.0085
R^2	0.9932	0.9993

2. Antioxidant Activiyt

2. 1. DPPH· Radical Scavenging

DPPH· assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH·. This method is a widely used to evaluate the free radical scavenging ability of various samples. It is a stable nitrogen- centered free radical the color of which changes from violet to yellow upon reduction by eitherthe process of hydrogen- or electron- donation [30]. Fig. 1 shows that DPPH· scavenging activity ofextract increased with increasing of concentrations and at 30 µg/mL the *H. tomentosum* is able to scavenge until 90% of DPPH· radical. The EC_{50} value of this extract is 9.32±0.96 µg/mL. Our value is in accordance with Bouratoua et al.[18], who reported that butanolic extract of *H. tomentosum* aerial part shows a potent DPPH· scavenging with EC_{50} = 14 µg/mL, this result may be justified by the high content of polyphenols and flavonoids. Trolox shows also a high DPPH· scavenging activity with EC_{50} value of 4.60 ±0.15 µg/mL; the same value was reported by Boudoukha et al.[31]

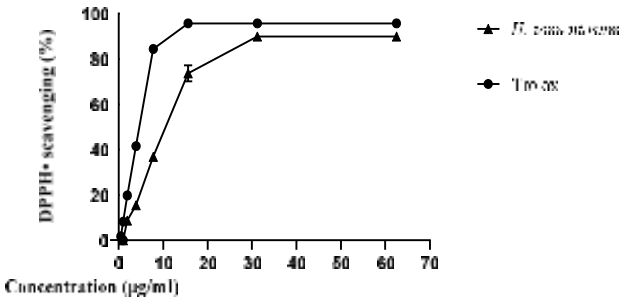

Figure 1: DPPH· scavenging activity of *H. tomentosum* leaves aqueous extract and Trolox. Values are means ± SD (n=3).

2. 2. Hydrogen Peroxide Scavenging Activity

The human exposure to H_2O_2 is very high, because of its incorporation into personal care products as bleaching agent or disinfectant. In addition, H_2O_2 can also be generated *in vivo* due to the activities of some enzymes such as superoxide dismutase. It has the ability to cross the cell membrane, thereby causing the oxidationof a number of compounds in the cytosol [32]. Fig. 2 shows that aqueous extract of *H. tomentosum* exerted a considerable dose dependent hydrogen peroxide scavenging activity with EC_{50}=3.13±0.49 µg/mL. This activity is close to that obtained by standard ascorbic acid (EC_{50}=1.23± 0.09 µg/mL). The potential scavenging activity is distributed to the hydroxyl groups in the aromatic ring of the phenolic components [33]. This group can act as proton donor thus it neutralizes the ROS. It donates the hydrogen to the hydrogen peroxide and cleavesit into water molecules [34].

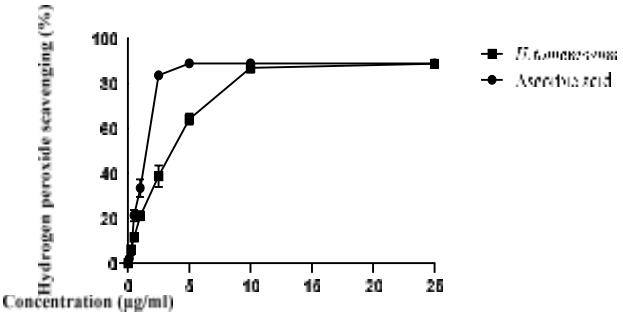

Figure 2: Hydrogen peroxide scavenging activity of *H. tomentosum* aqueous extract and ascorbic acid. Data represent means ± SD (n=3).

2. 3. Reducing Power Activity

The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers. The reducing power assay isoften used to evaluate the ability of the natural antioxidant to donate an electron [35]. In this assay, the presence of antioxidants in the samples would result in the reducing of ferric ion(Fe^{3+}) to ferrous ion (Fe^{2+}) by donating an electron. The amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance indicates an increase in reductive ability [30]. Fig. 3 shows the dose-response curves for the reducing power of extract and BHT, a standard used in this test. Reducing power of the extract increases with the increase of its concentrations. *Hypericum tomentosum* exercisesa powerful reducing ability which is close to the activity of BHT with EC_{50} values of 19.64±0.01µg/mL and 14.26±0.02µg/mL, respectively. This property is probably due to the presence of active components with hydroxyl groups that act as reductants [36] giving them a reducing power and they can be used as electron donors [37].

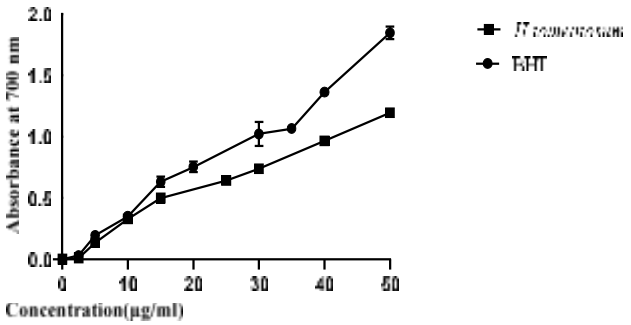

Figure 3: Reducing power capacity *of Hypericum tomentosum* and BHT. The values are expressed as means ± SD (n= 3).

3. 2. 4. Ferrous Ion Chelating

Metal ion chelating capacity plays a significantrole in antioxidant mechanisms by inhibition ROS formation and radical production [14]. *H. tomentosum* inhibited the formation of Fe^{2+} - ferrozine complex in a dose dependent manner (Fig. 4).

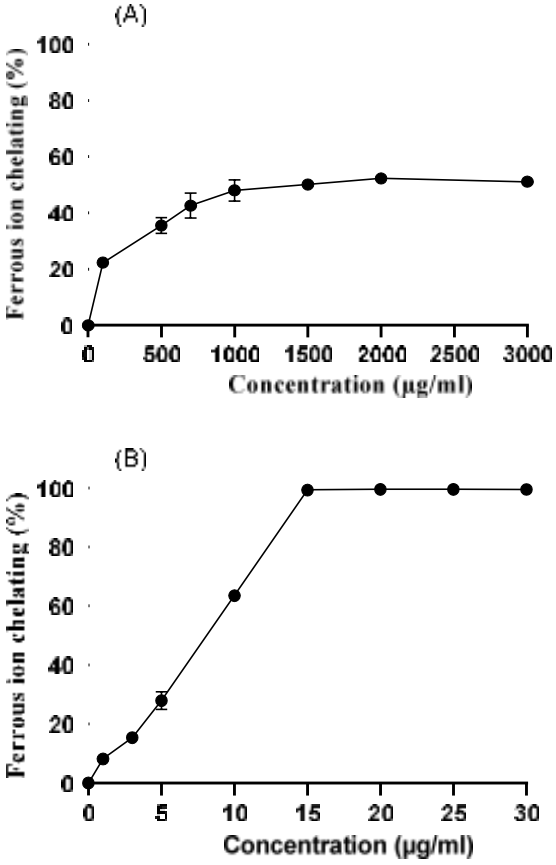

Figure 4: Ferrous ion chelating activity of *H. tomentosum* aqueous extract (A) and EDTA (B). The results are expressed as means ± SD (n=3).

Therefore, this activity was less important than EDTA which exerted a highest chelating activity. This results probably due to the richness of extract by flavonoids with weak chelating activity, it has been reported that some flavonoids, such as naringin, pelargonidin, phloridzin, and hesperitin had no chelating activity [38]. Fe2+ catalyzes the conversion of H2O2 into a highly reactive and damaging hydroxyl radical, a reaction known Fenton's reaction. Hence, *H. tomentosum* canblock this reaction by their potent ability to scavenge hydrogen peroxide proved above.

2. 5. Anti-Hemolytic Activity

Hemolysis of erythrocytes by AAPH serves as an *ex vivo* model for the oxidation damage of biological membranes [39]. The aim of this testwas focused on the ability of extract to prevent hemolysis caused by aqueous peroxyl radicals generated by thermal decomposition of AAPH at 37°C [40]. This hemolysis was followed continuously at 630 nm to verify changes in the turbidity of erythrocytes. Fig. 5 shows that *H. tomentosum* aqueous extract protect erythrocyte against hemolysis in a concentration dependent manner proving efficiency to scavenge radical species in the medium before they attack the erythrocytes.

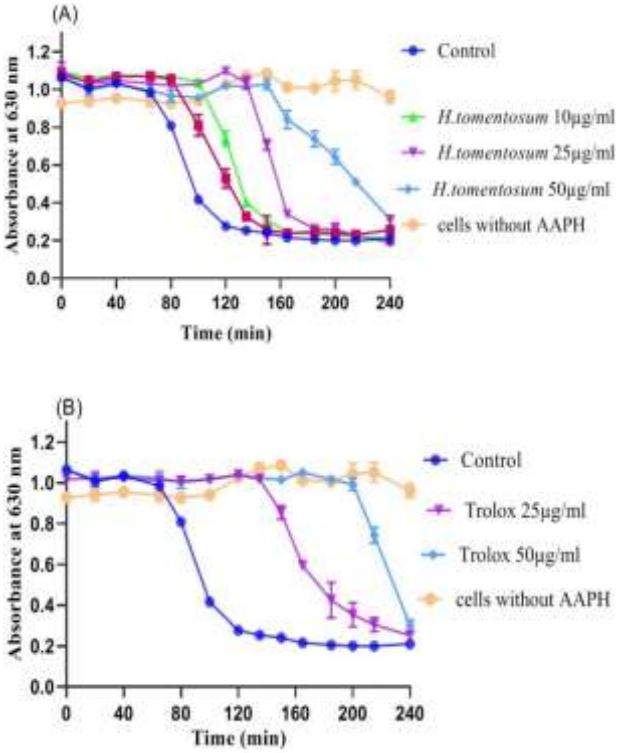

Figure 5: Changes in turbidity of erythrocytes samples during 4 hours in response to AAPH following incubation with *H. tomentosum* aqueous extract (A) and standard Trolox (B). Recording was started immediately following the addition of AAPH. Values are means ± SD (n = 3).

The results show a significant (*P*<.001) anti-hemolytic activity at the concentrations of 5, 10, 25 and 50 µg/mL, with half hemolysis time (HT_{50}) of 110.5±2.04 min, 123.5±2.09 min, 152.1±2.18 min and 206.7±2.31 min, respectively (Fig. 6). These results are close to those obtained by standard, Trolox. Likely, this anti-hemolytic activity is due to the richness of extract on flavonoids which enhance erythrocytes' resistance against free radical species, mainly by their capacity to capture them,by donating electrons and quenching radicals formed in the aqueous phase before they can damage the erythrocytes' membrane [41].

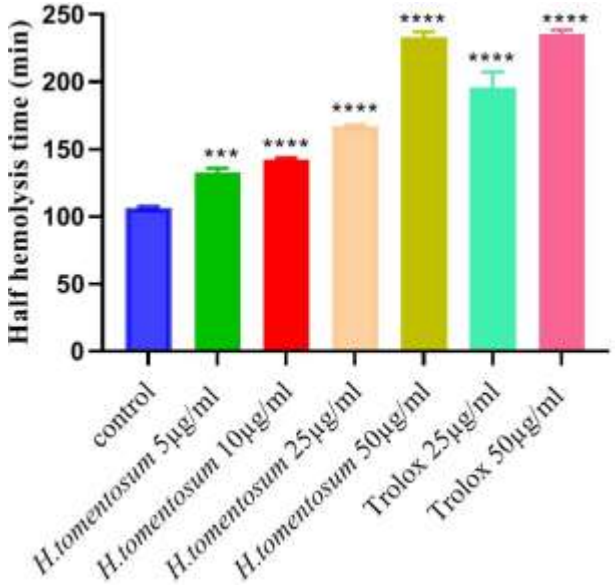

Figure 6: Half hemolysis time of *H.tomentosum* aqueous extract and Trolox. Data are expressed as mean ± SD. *P*< .001.

CONCLUSION

This study first focused on *in vitro* antioxidant activity of *Hypericum tomentosum*, which showed that aqueous extract of leaves of this plant exhibits a high antioxidant activity and prevents against erythrocytes hemolysis. These effectsmay be due to their high content of phenolics and flavonoids compounds. Hence, *H. tomentosum* could be a promising potential source of natural antioxidant for the prevention and/or treatment of oxidative stress-related diseases.

AKNOWLEDGMENTS

The authors are thankful to the Algerian Ministryof High Education for providing facilities to carry out the research work and we would like also to thank Prof. Hocine LAOUER (Laboratory of Valorization of Natural Biological Resources, University of Setif 1, Algeria) for the identification of the plant material.

COMPETING INTERESTS

The authors declare that they have no conflicts of interest

FUNDING

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS).

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors 'Author LA' designed the study, performed work, wrote the protocol, and wrote the first draft of the manuscript. 'Author MD' and 'Author KS' managed the analyses of the study. 'Author HB' and 'Author SA' designed the study, supervised the laboratory work. All authors read and approved the final manuscript.

REFERENCES

[1] Nemmiche S. Oxidative Signaling Response to Cadmium Exposure. Toxicological Sciences. 2016; kfw222.

[2] Dramane P, Yhi-pênê NJ, Adama H. Antioxidant and Anti-Inflammatory Activities of Caralluma Acutangula (Decne.) N.E.Br Extracts, a Medicinal Plant from Burkina Faso. Journal of Drug Delivery and Therapeutics. 2019; 9(6):155-61.

[3] Gupta N, Yadav KK, Kumar V, Kumar S, Chadd RP, Kumar A. Trace elements in soil-vegetables interface: Translocation, bioaccumulation, toxicity and amelioration - A review. Science of the Total Environment. 2019; 651:2927-42.

[4] Jagoda SV, Dixon KM. Protective effects of 1, 25 dihydroxyvitamin D_3 and its analogs on ultraviolet radiation-induced oxidative stress: a review. Redox Report. 2020; 25(1):11-6.

[5] Aouacheri O, Saka S. Cytoprotective Effects of *Zingiber officinale* Against the Oxidative Stress Induced by Lead Acetate Toxicity in Rats. Phytothérapie. 2021; 19(5-6):297-305.

[6] Song P, Zou MH. Roles of Reactive Oxygen Species in Physiology and Pathology. In: Wang H, Patterson C, éditeurs. Atherosclerosis [Internet]. Hoboken, NJ: John Wiley & Sons, Inc; 2015 [cité 9 mars 2022]. p. 379-92. Disponible sur: https://onlinelibrary.wiley.com/doi/10.1002/9781118828533.ch30

[7] Boussif A, Rolas L, Weiss E, Bouriche H, Moreau R, Périanin A. Impaired intracellular signaling, myeloperoxidase release and bactericidal activity of neutrophils from patients with alcoholic cirrhosis. Journal of Hepatology. 2016; 64(5):1041-8.

[8] Schmidt HM, Kelley EE, Straub AC. The impact of xanthine oxidase (XO) on hemolytic diseases. Redox Biology. 2019; 21:101072.

[9] El-Beltagi HS, Mohamed HI. Reactive Oxygen Species, Lipid Peroxidation and Antioxidative Defense Mechanism. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2013; 41(1):44.

[10] Demidchik V. Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environmental and Experimental Botany. 2015;109:212-28.

[11] Baudin B. Marqueurs d'oxydation desacides nucléiques. Revue Francophone des Laboratoires. 2020; (522):56-61.

[12] Favier A. Stress oxydant et pathologies humaines. Annales Pharmaceutiques Françaises. 2006;64(6):390-6.

[13] Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clinical Interventions in Aging. 2018; 13:757-72.

[14] Gulcin İ, Alwasel SH. Metal Ions, Metal Chelators and Metal Chelating Assay as Antioxidant Method. Processes. 2022; 10(1):132.

[15] Miara MD, Bendif H, Rebbas K, Rabah B, Hammou MA, Maggi F. Medicinal plants and their traditional uses in the highland region of Bordj Bou Arreridj (Northeast Algeria). Journal of Herbal Medicine. 2019; 16:100262.

[16] Benabdallah A, Boumendjel M, Aissi O, Rahmoune C, Boussaid M, Messaoud C. Chemical composition, antioxidant activity and acetylcholinesterase inhibitory of wild Mentha species from northeastern Algeria. South African Journal of Botany. 2018; 116:131-9.

[17] Henzelyová J, Čellárová E. Modulation of naphthodianthrone biosynthesis in hairy root-derived *Hypericum tomentosum* regenerants. Acta Physiologiae Plantarum. 2018; 40:82.

[18] Hosni K, Msaâda K, Ben Taârit M, Ouchikh O, Kallel M, Marzouk B. Essential oil composition of Hypericum perfoliatum L. and *Hypericum tomentosum* L. growing wild in Tunisia. IIndustrial Crops and Products. 2008; 27(3):308-14.

[19] Rouis Z, Laamari A, Abid N, Elaissi A, Cioni P.L, Flamini G, Aouni M. Chemical composition and larvicidal activity of several essential oils from hypericum species from Tunisien. Parasitology Research. 2012.

[20] Bouratoua A, Touafek O, Kabouche A, Zerizer S, Kabouche Z. Components and Antioxidant and Immunomodulatory Activities of *Hypericum tomentosum* subsp. pubescens. Chemistry of Natural Compounds. 2016; 52(5):893-5.

[21] Napoli E, Siracusa L, Ruberto G, Carrubba A, Lazzara S, Speciale A, et al. Phytochemical profiles, phototoxic and antioxidant properties of eleven Hypericum species – A comparative study. Phytochemistry. 2018; 152:162-73.

[22] Gülçin İ, Bursal E, Şehitoğlu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and Chemical Toxicology. 2010;48(8-9):2227-38.

[23] Messaoudi D, Bouriche H, Demirtas I, Senator A. Phytochemical Analysis and Hepatoprotective Activity of Algerian Santolina chamaecyparissus L. Extracts. Annual Research & Review in Biology. 2018; 25(2):1-12.

[24] Kada S, Bouriche H, Senator A, Demirtaş I, Özen T, Çeken Toptanci B, et al. Protective activity of *Hertia cheirifolia* extracts against DNA damage, lipid peroxidation and protein oxidation. Pharmaceutical Biology. 2017; 55(1):330-7.

[25] Que F, Mao L, Pan X. Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. Food Research International. 2006; 39(5):581-7.

[26] Kherbache A, Senator A, Laouicha S, Al-Zoubi RM, Bouriche H. Phytochemical analysis, antioxidant and anti-inflammatory activities of *Helichrysum stoechas* (L.) Moench extracts. Biocatalysis and Agricultural Biotechnology. 2020; 29:101826.

[27] Le K, Chiu F, Ng K. Identification and quantification of antioxidants in Fructus lycii. Food Chemistry. 2007; 105: 353–363.

[28] Meziti H, Bouriche H, Kada S, Demirtas I, Kizil M, Senator A. Phytochemical analysis, and antioxidant, anti-hemolytic and genoprotective effects of *Quercus ilex* L. an

Pinus halepensis Mill methanolic extracts. Journal of Pharmacy & Pharmacognosy Research. 2019; 7 (4): 260-272.

[29] Zubrická D, Mišianiková A, Henzelyová J, Valletta A, De Angelis G, D'Auria FD, et al. Xanthones from roots, hairy roots and cell suspension cultures of selected Hypericum species and their antifungal activity against Candida albicans. Plant Cell Reports. 2015; 34(11):1953-62.

[30] Fathi H, Ebrahimzadeh MA. Antioxidant and free radical scavenging activities of *Hypericum perforatum* L. (st. John's wort). 2013; 3:5.

[31] Boudoukha C, Elmastas M, Aksit H. Antioxidant capacity and phenolic content of *Santolina chamaecyparissus* L. methanol extract. International Journal of Green Pharmacy. 2019; 13 (3):269-267.

[32] Lateef A, Ojo SA, Elegbede JA, Azeez MA, Yekeen TA, Akinboro A. Evaluation of SomeBiosynthesized Silver Nanoparticles for Biomedical Applications: Hydrogen Peroxide Scavenging, Anticoagulant and Thrombolytic Activities. Journal of Cluster Science. 2017; 28(3):1379-92.

[33] Farahmandfar R, Esmaeilzadeh Kenari R, Asnaashari M, Shahrampour D, Bakhshandeh T. Bioactive compounds, antioxidant and antimicrobial activities of *Arum maculatum* leaves extracts as affected by various solvents and extraction methods. Food Science & Nutrition. 2019; 7(2):465-75.

[34] Prahadeesh N, Sithambaresan M, Mathiventhan U. A Study on Hydrogen Peroxide Scavenging Activity and Ferric Reducing Ability of Simple Coumarins. Emerging Science Journal. 2018; 2(6):417.

[35] Ameni D, Baghiani A, Boumerfeg S, Dahamna S, Khennouf S, Zarga MHA, et al. Phytochemical profiles, antioxidant capacity and protective effect against AAPH-induced mouse erythrocyte damage by *Daphne gnidium* L. Shoots extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7(11):9.

[36] Sharma P, Ravikumar G, Kalaiselvi M, Gomathi D, Uma C. *In vitro* antibacterial and free radical scavenging activity of green hull of Juglans regia. Journal of Pharmaceutical Analysis. 2013;3(4):298-302.

[37] El Moussaoui A, Jawhari FZ, Almehdi AM, Elmsellem H, Fikri Benbrahim K, Bousta D, et al. Antibacterial, antifungal and antioxidant activity of total polyphenols of *withania frutenscens L.* Bioorganic chemistry; 2019;73:10337.

[38] Van Acker SABE, Van Den Berg D jan, Tromp MNJL, Griffioen DH, Van Bennekom WP, Van Der Vijgh WJF, et al. Structural aspects of antioxidant activity of flavonoids. F Free Radical Biology and Medicine. 1996; 20(3):331-42.

[39] Nuruki Y, Matsumoto H, Tsukada M, Tsukahara H, Takajo T, Tsuchida K, et al. Method to Improve Azo-Compound (AAPH)-Induced Hemolysis of Erythrocytes for Assessing Antioxidant Activity of Lipophilic Compounds. Chemical and Pharmaceutical Bulletin. 2021;69(1):67-71.

[40] Elizondo-Luévano JH, Castro-Ríos R, López-Abán J, Gorgojo-Galindo O, Fernández-Soto P, Vicente B, et al. Berberine: A nematocidal alkaloid from Argemone mexicana against Strongyloides venezuelensis. Experimental Parasitology. 2021; 220:108043.

[41] Gonçalves AC, Bento C, Silva BM, Silva LR. Sweet cherries from Fundão possess antidiabetic potential and protect human erythrocytes against oxidative damage. Food Research International. 2017; 95:91.

الملخص

تهدف هذه الدراسة إلى تقييم الفعالية المضادة للإلتهاب و المضادة للأكسدة للمستخلصين المائي (Aq.E) و الميثانولي (Met.E) لأوراق نبتة *Hypericum tomentosum*. بين التقدير الكمي للمركبات الفينولية أن المستخلص الميثانولي لهذه النبتة يحتوي على أعلى كمية من عديدات الفينول، الفلافونويدات والدباغ مقارنة بالمستخلص المائي. أظهر تحليل HPLC وجود العديد من الفلافونويدات والأحماض الفينولية حيث سجل الكرسترين والإيبيكاتيشين أعلى نسبة من بين الفلافونويدات في حين سجل كل من حمض الكينيك وحمض الكلوروجينيك أعلى تركيز من بين الأحماض الفينولية. تم تقدير النشاطية المضادة للأكسدة للمستخلص المائي و الميثانولي من خلال تحديد أنشطة الكسح ل DPPH•، OH• و H_2O_2. أظهرت النتائج أن المستخلصين المائي و الميثانولي ثبطا جذور DPPH• بقيم IC_{50} مساوية مساوية ل 9,32 و 5,85 ميكروغرام/ مل على التوالي، كما ثبطا جذور OH• بقيم IC_{50} مساوية ل 338,8 و 377,6 ميكروغرام/ مل على التوالي، وكانت قيم IC_{50} مع H_2O_2 3,13 و2,07 ميكروغرام/ مل على التوالي. من جهة أخرى، أظهر كلا المستخلصين قدرة بالغة على إرجاع الحديد وقدرة على تثبيط أكسدة الليبيدات من خلال التأثير الوقائي الكبير ضد تحلل كريات الدم الحمراء الناجم عن الجذور الحرة وأيضا من خلال تثبيط أكسدة حمض اللينولوييك بنسبة 77,48 % للمستخلص المائي و بنسبة 29,43 % للمستخلص الميثانولي، في حين كانت قدرة المستخلصين على إستخلاب أيونات الحديد ضعيفة. بينت نتائج النشاطية المضادة للإلتهاب أن معالجة الفئران ب 1 مغ/مل من المستخلص المائي و الميثانولي إلى تثبيط معنوي (p<0,001) في هجرة الخلايا المتعادلة بنسبة 38,53 % و 47,5 % على الترتيب، كما أدى التطبيق الموضعي لكلا المستخلصين المائي و الميثانولي في نموذج وذمة الأذن الناجمة عن زيت الكروتون عند الفئران إلى تقليل الوذمة بنسبة 68,75 % و 67 % على التوالي، في حين أظهرت معالجة الفئران بتركيز 200 و 400 مغ/ كغ من كلا المستخلصين عن طريق الفم فعالية ضعيفة في تقليل وذمة الرجل المحدثة بالكراجينين. أبدى كلا المستخلصين بنفس التراكيز قدرة على تثبيط الألم المحدث بحمض الأستيك. أثبت كلا المستخلصين نشاط مضاد للالتهاب من خلال اختبارات تقييم تأثيرهما على وظائف الخلايا البيضاء المتعادلة البشرية، حيث أظهرا تثبيطًا كبيرًا و متعلق بالتركيز على نشاط كل من إنزيم الإيلاستاز و إنزيم الميالوبيروكسيداز، كما بين كلا المستخلصين فعالية معتبرة (p<0,05) على تثبيط الخلايا المتعادلة على قتل *Escherichia coli* ومع ذلك ، في اختبار البلعمة الخلوية لخلايا خميرة *Candida albicans* ، أبدى المستخلصان اللذان تمت دراستهما تثبيطا طفيفا وغير معنوي لنسبة البلعمة.

الكلمات المفتاحية: مضادات الإلتهاب، مضادات الأكسدة، *Hypericum tomentosum*، الفلافونويدات، عديدات الفينول.

Abstract

This study aims to evaluate the anti-inflammatory and antioxidant activities of aqueous (Aq.E) and methanolic (Met.E) extracts of *Hypericum tomentosum* leaves. The quantitative assessment of phenolic compounds showed that Met.E extract of this plant contains the highest amount of polyphenols, flavonoids and tannins compared to Aq.E. HPLC analysis showed the presence of many flavonoids and phenolic acids, quercetrin and epicatechin recorded the highest amount among flavonoids, while quinic acid and chlorogenic acid had the highest concentration among the phenolic acids. The antioxidant activity of Aq.E and Met.E was estimated *in vitro* by determining the scavenging activity of DPPH•, OH• and H_2O_2. The results show that both Aq.E and Met.E inhibited DPPH• with IC_{50} values of 9.32 and 5.85 µg/mL, respectively and they inhibited OH• with IC_{50} equal to 338.8 and 377.6 µg/mL and the values of IC_{50} with H_2O_2 were 3.13 and 2.07 µg/mL, respectively. Both extracts also showed a great ability to reduce ferric ion and inhibit lipid oxidation, through the protective effect against the lysis of red blood cells caused by radicals attack and through prevention of linoleic acid oxidation by inhibition levels of 77.48 % with Aq.E and 29.43 % with Met.E, while the ability of the two extracts to chelate ferrous ions was weak. The results of anti-inflammatory activity demonstrated that treating mice with 1 mg/mL of Aq.E and Met.E extracts significantly (p<0.001) inhibited neutrophils migration by 38.53% and 47.5%, respectively. Topical application of both Aq.E and Met.E to mice, in the ear edema model induced by croton oil, reduced edema by 68.75% and 67%, respectively. While orally treatment of mice with a concentration of 200 mg/kg and 400 mg/kg of both extracts showed a weak effectiveness in reducing paw edema induced by carrageenan. The same concentrations proved an ability to inhibit pain caused by acetic acid. Both extracts showed anti-inflammatory activity through tests evaluating the effects of the studied extracts on human neutrophils functions. They exerted a big inhibition and dose-dependent manner elastase and myeloperoxidase activities. The two extracts were shown to be able to inhibit significantly (p<0.05) bactericidal activity exerted on *Escherichia coli*. However, in assay of cellular phagocytosis of *Candida albicans* cells by human neutrophils, the studied extracts showed slight and non-significant inhibition of phagocytosis.

Keywords: Anti-inflammatory, Antioxidant, *Hypericum tomentosum*, Flavonoids, Polyphenols.

Résumé

Cette étude vise à évaluer l'activité anti-inflammatoire et anti-oxydante de l'extrait aqueux (E.Aq) et méthanolique (E.Met) des feuilles de la plante *Hypericum tomentosum*. L'évaluation quantitative des composés phénoliques montre que l'E.Met de cette plante contient des quantités plus élevées en polyphénols, flavonoïdes et en tanins par rapport à l'E.Aq. L'analyse HPLC a montré la présence de nombreux flavonoïdes et acides phénoliques, la quercitrine et l'épicatéchine ont enregistré la concentration la plus élevée parmi les flavonoïdes, tandis que l'acide quinique et l'acide chlorogénique existent avec des concentrations les plus élevées parmi les acides phénoliques. L'activité anti-oxydante de E.Aq et E.Met a été estimée *in vitro* par la détermination de l'activité scavenger de DPPH•, de l'OH• et de l'H_2O_2. Les résultats ont montrés que l'E.Aq et l'E.Met ont inhibé le DPPH• avec les valeurs d'IC_{50} de l'ordre de 9,32 et 5,85 µg/mL, respectivement. Ils ont inhibé aussi le OH• avec des IC_{50} de 338,8 et 377,6 µg/mL, respectivement. Les valeurs des IC_{50} avec l'H_2O_2 sont égales à 3,13 et 2,07 µg/mL, respectivement. Les deux extraits ont également montré une forte capacité à réduire le fer ferrique et à inhiber l'oxydation des lipides, à travers la prévention contre la lyse des globules rouges causée par l'attaque radicalaire et via l'empêchement de l'oxydation de l'acide linoléique avec des niveaux d'inhibition de 77,48 % avec l'E.Aq et de 29,43 % avec l'E.Met. Bien que les deux extraits aient un faible effet chélateur des ions ferreux. Les résultats de l'activité ant-iflammatoire montrent que le traitement des souris avec 1 mg/mL de l'E.Aq et l'E.Met inhibe significativement (p<0,001) la migration des neutrophiles avec des taux de 38,53 % et 47,5 %, respectivement. L'application topique de l'E.Aq et l'E.Met chez les souris dans le modèle de l'œdème de l'oreille induit par l'huile de croton a réduit l'oedème avec des taux d'inhibition de 68,75 % et 67 %, respectivement. Tandis que le traitement par voie orale des deux extraits à 200 mg/kg et 400 mg/kg chez les souris ait montré un faible effet sur la réduction de l'œdème de la patte causé par la carragénane, les mêmes concentrations de l'extrait aqueux et méthanolique ont exercé un effet inhibiteur sur la douleur causée par l'acide acétique. En évaluant l'effet des extraits étudiés sur les fonctions des neutrophiles humains, les deux extraits ont montré une grande inhibition dose-dépendante de l'activité de l'élastase et de la myéloperoxydase. Ces deux extraits se sont révélés capables d'inhiber significativement (p<0,05) l'activité bactéricidie exercé sur *Escherichia coli*. Cependant, dans le test de la phagocytose de la levure *Candida albicans*, les extraits étudiés ont montré une inhibition légère et non significative de la phagocytose.

Mots clés: Anti-inflammatoire, Antioxydant, flavonoïdes, *Hypericum tomentosum*, polyphenols.