الجمهورية الجزائرية الديمقراطية الشعبية
Democraticand Popular Republicof Algeria

وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research

جامعة مولاي الطاهر، سعيدة
University of Saida “Dr Tahar Moulay”

N° Ordre

كلية العلومالطبيعية والحياة
Faculty of Natural and Life Science

قسم البيولوجيا
Departmentof Biology

**Dissertation for obtaining the master’s degree in Biological Sciences**

**option:**Biochemistry

Theme

# Antioxidant activity evaluation of three medicinal plants of the *Asteraceae* family and their binary combination

presented by: **Ms.SouhilaBENALI**

Supportedon:June 22, 2025

Before the jury composed of:

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| **President** | Dr. Hayet DIDAOUI | MCA | University of Saida -Dr Tahar Moulay |
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| **Supervisor** | Dr Amel SIDI IKHLEF | MCB | University of Saida -Dr Tahar Moulay |

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## *Acknowledgments*

*As a preamble to this thesis, I would like to thank the good Lord Almighty powerful for granting me the opportunity to reach this stage of knowledge, and for giving me the courage and patience to accomplish this modest work.*

*I thank my supervisors Dr.* ***SidiIkhlef Amel*** *for agreeing to supervise and carry out my work.*

*I would like to sincerely thank the president Dr.* ***Didaoui Hayet*** *and the examiner* ***Arabi Wafaa*** *for their time,efforts and valuable and remarks that greatly enriched this masterè`s thesis.*

*I extend my sincerest thanks to Mr.* ***Otman****, head of the biology laboratory.*

*My gratitude also goes to all the staff and the* ***teachers*** *of the Faculty of Natural and Life Science.*

*Dedication*

*I dedicate this model work and my deep gratitude.*

*To all those who sacrificed to provide me with the conditions conducive to my success:*

*To my dear mother '**Rekaia**', to whom I owe my success, for the education she provided me, with all the means and at the cost of all the sacrifices she made for me, for the sense of duty she taught me over the past 17 years.*

*To my first teacher in this life, your words will remain stars that guide me today, tomorrow, and forever. Dear father **"Djaloul,"** may God protect you and grant you a place in His vast paradise.*

*To my dear brothers: '**Abderrahman', 'Choaib**', and '**Mohammed'.** to my dear sisters **"Kawtar," and "Alaa Zineb."***

*To my dear friends: "Sara," "**Malika," "Amina," "Malika M.," "Imane," "Soumia," "Fatima," "Asmaa**," and "**Chaimaa."***

## Abbreviations list

**AP:** *Anacyclus pyrethrum*

**BC**: Before Christ

**CI**: *Cichorium intybus*

**COX**: Cyclooxygenase

**DPPH:** 2,2-diphényl-1-picrylhydrazyle

**$H_2SO_4$ :** Sulfuric acid

**HepG2:** hepatitis G2

**HT 29:**adherent epithelial cell line

**IC:** inhibitory concentration

**MAYV:** Mayaro virus

**MI:**Myocardial infarction

**NaOH:** sodium hydroxide

**NFLD:** nonalcoholic fatty liver disease

**$PGE_2$:** prostaglandin $E_2$

**ROS**: Reactive Oxygen Species

**SM**: *Silybum marianum*

**TNF**: Tumor necrosis factor

**UVA :** Ultraviolet A rays

## Tables List

# FiguresList

**Abstract**

Plants with medicinal properties play an increasingly important role in food and pharmaceutical industries for their functions on disease prevention and treatment. This study characterizes the qualitative Phytochemical contents and antioxidant activity of three plants, including the roots of *Anacyclus pyrethrum*(AP), stemsof*Cichorium intybus*(CI), as well as the seeds of *Silybum marianum*(SM). in addition, we studied the antioxidant effects of their binary mixtures.

The analysis of the phytochemicals qualitatively showed the existence of alkaloids in the three plants, flavonoids and catcholic tannins in CI and SM. in addition we found the anthocyanins and Starch in CI and SM extracts respectively. However, Coumarins, Reducing Compounds, Saponins and gallic tannins were absent in all plants. Results of antioxidant activity revealed that the hydro-methanolic roots and stems extracts of AP and CI respectively, displayed the highest antiradical effect with IC50 393 and 392 µg/ml respectively, however SM extract seeds presented low antioxidant activity with IC50 1500.2 µg/ml. in addition we found that AP and CI increases the antiradical effect of SM. While the mixture of the extract AP and CI causes a decrease in antioxidant activity compared to the two individual plants.

Our study suggests antioxidant interactions in a mixture can influence its antioxidant activity positively or negatively. Further studies are desired on the identification of biochemical compounds of these plants and in vivo studies should be performed to confirm the results obtained so far.

**Keywords:** *phytochemicals, Antioxidant activity;Anacyclus pyrethum;Cichorium intybus;Silybum marianum; antioxidant activity;; binary mixtures.*

**Résumé**

Les plantes aux propriétés médicinales jouent un rôle de plus en plus important dans les industries agroalimentaire et pharmaceutique, en raison de leur rôle dans la prévention et le traitement des maladies. Cette étude caractérise le contenu phytochimique qualitatif et l'activité antioxydante de trois plantes, dont les racines d'*Anacyclus pyrethrum* et les tiges de *Cichorium intybus*, ainsi que les graines de *Silybum marianum*. Nous avons également étudié les effets antioxydants de leurs mélanges binaires.

L'analyse des composés phytochimiques a montré qualitativement l'existence d'alcaloïdes dans les trois plantes, de flavonoïdes et de tanins catholiques dans le CI et le SM. De plus, nous avons trouvé des anthocyanes et de l'amidon dans les extraits de CI et de SM respectivement. Cependant, les coumarines, les composés réducteurs, les saponines et les tanins galliques étaient absents dans toutes les plantes. Les résultats de l'activité antioxydante ont révélé que les extraits hydro-méthanoïques d'AP et de CI présentaient l'effet antiradicalaire le plus élevé avec une CI50 de 393 et 392 µg/ml respectivement, tandis que l'extrait de SM présentait une faible activité antioxydante avec une CI50 de 1500,2 µg/ml. De plus, nous avons constaté que l'AP et le CI augmentent l'effet antiradicalaire du SM. Alors que le mélange de l'extrait AP et CI provoque une diminution de l'activité antioxydante par rapport aux deux plantes individuelles.

Notre étude suggère que les interactions antioxydantes dans un mélange peuvent influencer son activité antioxydante positivement ou négativement, des études supplémentaires sont souhaitées sur l'identification des composés biochimiques de ces plantes et des études in vivo doivent être réalisées pour confirmer les résultats obtenus jusqu'à présent.

**Mots-clés :***composés phytochimiques; activité antioxydante*; *Anacyclus pyrethrum*; *Cichorium intybus*; *Silybum marianum*; *mélanges binaires*.

# ملخص

تلعب النباتات ذات الخصائص الطبية دورًا متزايد الأهمية في الصناعات الغذائية والدوائية نظرًا لوظائفها في الوقاية من الأمراض وعلاجها. تُقيّم هذه الدراسة المحتوى الكيميائي النباتي النوعي ونشاط مضادات الأكسدة في ثلاثة نباتات، بما في ذلك جذور نبات Anacyclus pyrethrum (AP)، وسيقان نبات Cichorium intybus (CI)، بالإضافة إلى بذور نبات Silybum marianum (SM). بالإضافة إلى ذلك، درسنا التأثيرات المضادة للأكسدة لمزائجها الثنائية.

أظهر تحليل المواد الكيميائية النباتية نوعيًا وجود alkaloids في النباتات الثلاثة، flavonoids و catcholic tannins في مستخلصي CI وSM. بالإضافة إلى ذلك، وجدنا anthocyanins والنشا في مستخلصي CI وSM على التوالي. ومع ذلك، كانت coumarins، والمركبات المختزلة، وsaponins, و gallic tannins غائبة في جميع النباتات. كشفت نتائج النشاط المضاد للأكسدة أن مستخلصات الجذور والسيقان الهيدرو-ميثانولية لـ AP وCI على التوالي، أظهرت أعلى تأثير مضاد للجذور مع IC50 393 و392 ميكروغرام/مل على التوالي، ومع ذلك أظهرت بذور مستخلص SM نشاطًا مضادًا للأكسدة منخفضًا مع IC50 1500.2 ميكروغرام/مل. بالإضافة إلى ذلك، وجدنا أن AP وCI يزيدان من التأثير المضاد للجذور لـ SM. بينما يتسبب خليط مستخلصي AP وCI في انخفاض في النشاط المضاد للأكسدة مقارنةً بالنباتين الفرديين.

تشير دراستنا إلى أن تفاعلات مضادات الأكسدة في خليط ما يمكن أن تؤثر على نشاطه المضاد للأكسدة إيجابًا أو سلبًا. ونرغب في إجراء المزيد من الدراسات لتحديد المركبات الكيميائية الحيوية لهذه النباتات، كما ينبغي إجراء دراسات حيوية لتأكيد النتائج التي تم الحصول عليها حتى الآن.

الكلمات المفتاحية: مواد كيميائيّة نباتية؛ نشاط مضاد الاكسدة؛ Anacyclus pyrethrum; Cichorium intybus; silybum marianum; خلائط ثنائية

# Table of Contents

# PART I. INTRODUCTION

# Introduction

Medicinal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries around the world. Empirical knowledge about their beneficial effects was transmitted over the centuries within human communities**(Marrelli, 2021).**

*Anacyclus pyrethrum*(AP) is mainly distributed in Mediterranean region and India. traditionally is used for its antiepileptic and antirheumatic properties. In addition, this plant showed different activities including antidiabetic, antioxidant and anticancer**(Manouze et al., 2019).***Cichorium intybus*(CI) is widely distributed in Asia and Mediterranean regions**(Piccolella et al., 2024),** it is traditionally used as antidiabetic,antibacterial, anti-inflammatory, diuretic, digestive and protectorby other disorders**(Ceccanti et al., 2022).***Silybum marianum*(SM) is considered an important and ancient therapeutic plant. It is native to southern areas of Europe, Northern Africa, South and North America, and Australia, it is used to cure liver diseases and is beneficial for lactating mothers**(Javeed et al., 2022)**These medicinal properties are due to the presence of polyphenols, tannins, coumarins, sterols, triterpenes, alkaloids and some trace metals (**Ceccanti et al., 2022 ; Javeed et al., 2022).**

In the process of cellular oxygen reduction during ATP production, by-products known as reactive oxygen species (ROS) are formed in the human body. Among ROS, free radicals, such as $OH^\bullet$ or $O^\bullet_2$, can be distinguished. ROS can also come from exogenous sources such as smoking or environmental pollutants. The accumulation of ROS leads to damage to many cellular structures, which can result in the disruption of cellular homeostasis. Hence, when there is an imbalance between the ROS and endogenous antioxidants, exogenous antioxidants taken with food can be an effective factor in the fight against free radicals. Excellent alternative sources of natural antioxidants are plant products, such as fruits, vegetables and herbs. They contain several substances with antioxidant activity, such as vitamins A, C and E and carotenoids; phenolic compounds (phenolic acids, flavanols, flavones, catechins, anthocyanins and anthocyanidins, lignans and stilbenes); tannins; and coumarin**(Ulewicz-Magulska and Wesolowski, 2023).**

The aim of the work was to study the antioxidant activity of extracts obtained from the following medicinal plants: pellitory (*Anacyclus pyrethrum* L.), Chicory (*Cichorium intybus* L.), Milk thistle (*Silybum marianum* L.), and their binary mixtures.

# PART II. BIBLIOGRAPHIC SYNTHESIS

## II.1 Medicinal plants

### II.1.1 Historical use of medicinal plants

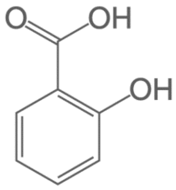
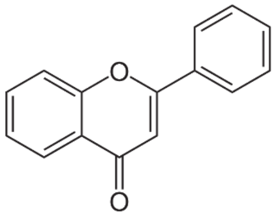
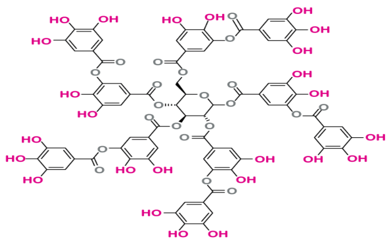
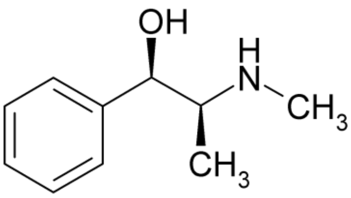
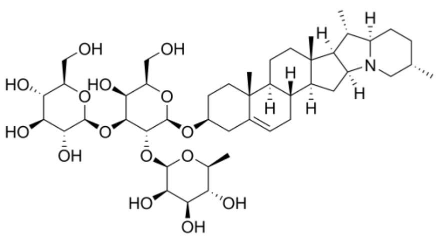
Over time, this knowledge was documented in ancient writings and medical manuscripts,detailing the healing properties of different plants and their specific uses. For example, intraditional Chinese medicine, the "Shennong Ben Cao Jing," a text dating back to around 200BC, lists hundreds of plants and their medicinal uses**.** With the advent of modern science, thestudy of medicinal plants became more systematic. Researchers began isolating and identifying the active compounds present in plants, as well as studying their mechanisms of action and clinical efficacy. This led to a better understanding of the scientific basis behind the traditional uses of medicinal plant. Today, research on medicinal plants continues within the framework of modern pharmacology, with growing interest in plants as potential sources of new drugs. Many modern medications are derived from chemical compounds found in plants, underscoring the ongoing importance of exploring the botanical wealth of our planet for medicine **(Šantić et al.,2017).**

### II.1.2 Phytochemistry of medicinal plants

Phenolic acids represent a significant class of phytochemicals found in plants, garneringconsiderable attention due to their antioxidant properties and potential health benefits**.** They aresynthesized within plants through the shikimate/phenylpropanoid pathway, highlighting theiressential role in plant metabolism and defense mechanisms. Phenolic acids are broadlycategorized into two main classes: benzoic acid derivatives and cinnamic acid derivatives.Examples include gallic acid, caffeic acid, ferulic acid, p-coumaric acid, and chlorogenic acidResearch suggests that phenolic acids may offer protective effects against degenerativediseases, owing to their antioxidant and anti-inflammatory properties. Their potential healthbenefits are extensively investigated in numerous studies. The bioavailability of phenolic acidsis crucial for understanding their physiological effects. They can be absorbed directly from thediet or indirectly through the metabolism of flavonoids by gut microbiota, highlighting thecomplex interplay between diet, gut health, and bioactive compounds **(Leong and Singhal, 2015).**

Flavonoids represent a diverse group of plant compounds renowned for their antioxidantand anti-inflammatory effects, often associated with various health benefits. Flavonoids areclassified into several subclasses, including flavanones, flavones, flavanols, and flavonols.They are abundantly found in foods such as apples, onions, red wine, grapes, citrus fruits, tea, berries, and olive oil. epidemiological evidence indicates that a higher intake of flavonoids is generally correlated with a reduced risk of cardiovascular diseases. However, intervention trialsusing pure flavonoid compounds remain scarce **(Kaushik et al., 2021).**

**Table 1:** Phytochemical structure of medicinal plants contents**(Kaushik et al., 2021).**

| **Chemical name** | **Chemical structure** |
|---|---|
| Phenolic acids |  |
| Flavonoids |  |
| Tannin |  |
| Alkaloids |  |
| Terpenoids |  |
| Saponins |  |

tannins represent a diverse array of polyphenolic compounds found in plants, offering astringent properties and potential health advantages, albeit with the caveat of potentially interfering with the absorption of specific nutrients **(Kaushik et al., 2021).**

Alkaloids emerge as nitrogen-containing compounds with diverse pharmacological activities, frequently found in medicinal plants and renowned for their therapeutic effects. Alkaloids constitute a broad group of naturally occurring chemical compounds characterized by the presence of basic nitrogen atoms within their structures. They are prevalent in various plants, with particular abundance noted in the Solanaceae (nightshade) family and are distinguished for their potent physiological effects on both humans and animals **(Sharma et al., 2020).**

Terpenoids emerge as a vast class of compounds with diverse biological activities, encompassing antimicrobial, anti-inflammatory, and antioxidant properties, prominently found in the essential oils of plants. Many terpenoids exhibit potent biological activities, ranging from antimicrobial and anti-inflammatory to antioxidant, anticancer, and immunomodulatory effects. Essential oils, characterized by their volatile and aromatic nature, serve as major repositories of terpenoids in plants **(Masyita el al., 2022).**

Saponins are a structurally diverse class of compounds occurring in many plant species, which are characterized by a skeleton derived from the 30-carbon precursor oxidosqualene to which glycosyl residues are attached. Traditionally, saponins are subdivided into triterpenoid and steroid glycosides, or into triterpenoid, spirostanol, and furostanol saponins. The oleanane skeleton is the most common saponin skeleton and is present in most orders of the plant kingdom. Carbohydrate chains of up to 18 monosaccharide residues can be attached to the saponin skeleton, most commonly at the C3 and/or C17 atoms. The diverse structures of saponins contribute to their wide range of biological activities and potential health benefits **(Vincken et al., 2007).**

## *II.2 Anacyclus pyrethrum*

### II.2.1 Presentation of plant

*Anacyclus pyrethrum* is a perennial plant belonging to the Asteraceae family. It is a species with a height of 40 to 60 cm and is characterized by numerous simple or small branching stems growing from the ground, and bearing leaves that are finely cut, delicate and pubescent. Its yellow-hearted flowers consist of white ray florets on the inside and purple on the outside. The roots are long, thick, fibrous, brown on the outside and white on the inside **(Figure 1).**All fruits (Achene) are bald or with a faint crown. The species bloom between May and August**(Elazzouzi et al., 2022).**It is found in North Africa, Arabia, Syria, Algeria, elsewhere in the mediterranean region and varieties of this drug are seen in some place of India such as Jammu and Kashmir, Bengal. It is imported to India from Algeria. They have slight aromatic smell and persistent pungent taste (The Wealth of India)**(Usmani et al., 2016).**

**Figure 1:** *Anacyclus pyrethrum* plant**(Yousef et al., 2021)**

### II.2.2 Taxonomic and chemical constituents

*Anacyclus pyrethrum* (L) is commonly known as African pyrethrum, akarkarha, tigendesste, and igendess. It is a species belonging to the family Asteraceae, which is indigenous to Morocco, Algeria, and Spain **(Jawhari et al., 2020).**

**Table 2:** Taxonomic Hierarchy of *Anacyclus pyrethrum* **(Usmani et al., 2016).**

| | |
|---|---|
| Kingdom | Plantae |
| Division | Spermatophyta |
| Subdivision | Angiosperms |
| Class | Dicotyledons |
| Subclass | Metachlamydae |
| Order | Companulatae |
| Family | Asteraceae |
| Genus | *Anacyclus* |
| Species | *Pyrethrum* |

Numerous chemical compositions of *A. pyrethrum* have been reported, with phytochemical screening of the roots, leaves, and flowers indicating the presence of alkaloids, reducing compounds, tannins, triterpenes, sterols, coumarins, sugars, and glycosides. The contents of flavonoids, total phenols, and polyphenols have been reported to be higher in flowers compared with those in leaves and roots. Its roots are abundant in alkaloids, whereas the aboveground part is rich in alkaloids, tannins, flavonoids, gums, and essential oils. Pellitorine, anacyclin, phenylethylamine, inulin, polyacetylenic amides I–IV, and sesamin are among the most significant compounds detected in the roots of *A. pyrethrum*. Volatile oilcontains various volatile oils, such as spathulenol, salvial-1-one, caryophyllene. Their content varies at different growth stages of plants, affecting plant aroma and biological activities. Non-volatile components contain various non-volatile compounds including alkaloids, phenolic compounds, and terpenoids. Alkaloids such as pellitorin exhibit anti-inflammatory and analgesic activities. Phenolic compounds, such as flavonoids, exert antioxidant and anti-inflammatory effects **(Tuersong et al., 2024).**

### II.2.3 Biological activities

Phenolic and flavonoid compounds are common constituents of *A. Pyrethrum*, and they exhibit various biological activities, including antioxidant and antimicrobial effects. The methanolic extract exhibited the most effective antimicrobial activity against *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, and Candida albicans.* Furthermore, *A. pyrethrum* has exhibited antimicrobial activity against *Bacillus*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Listeria*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, and *Candida genera* except for *Enterococcus faecalis* and *Salmonella typhimurium* **(Tuersong et al., 2024).**

*A. pyrethrum* exhibits aphrodisiac, sexual stimulation, and spermatogenic properties and can promote androgen production. Alkylamides, the principal alkaloids in *A. pyrethrum*, enhance testosterone secretion and may exert a role in alkylamide-induced hypothalamic stimulation, and it can enhance fertility, increase sperm count, and boost male sexual drive**(Tuersong et al., 2024).**

The antidiabetic activity of aqueous root extract of Anacyclus pyrethrum was evaluated in alloxan induced diabetic rats. The aqueous root extract of Anacyclus pyrethrum at a conc. of 150 and 300 mg/kg was orally administered to Alloxan induced diabetic rats. The prominent levels of blood glucose in the diabetic rats reverted to near normal after treatment with the aqueous root extract of *Anacyclus pyrethrum***(Usmani et al., 2016).**

Depression and cardiovascular diseases are combined to each other. It means when patient suffers from cardiovascular diseases then there is a risk of increasing the chances of depression and when it develops, cardiovascular risk is exacerbated further. *Anacyclus pyrethrum* shows significant results with forced swim test and tail suspension test. Agitation is a highly energy consuming and immobility is an energy conservative process. In addition, The ethanolic extract study of *Anacyclus pyrethrum* increases the brain cholinesterase level and possess the memory enhancing activity in scopolamine induced amnesia model by enhancing central cholinergic neurotransmission**(Rani et al., 2013).**

## II.3 *Cichorium intybus*

### II.3.1 Presentation of plant

The name of the common chicory plant (*Cichorium intybus* L.). The occurrence of this species was documented in Northern and Central Europe, Siberia, Turkey, Afghanistan, North and Central China, South America, South Africa, Ethiopia, Madagascar, India, Australia, New Zealand. *Cichorium intybus* L. is thus an important medical plant in all of Eurasia and some of Africa**(Janda et al., 2021).**

*Cichorium intybus* L. is an annual, biennial or perennial plant belonging to the family *Asteraceae* . It is winter hardy and usually reaches 20 to 150 cm in height. It forms a long and strong, spindly, thick, brown tapio root. The stem and vein are usually green, although they can take on an occasional red ring. *Cichorium intybus* can grow from many tall, empty, ribbed stems, each of which can be lifted or rising. It is stiff and has few leaves. At the bottom of the stem there are short, thick hairs, and at the top there are most branches. The buds are branched, stiff and contain no juice. The leaves of *C. intybus* are green, arranged in a

10–25 cm long rosette. Their shape is narrowly oval, oblong, lanceolate, usually pinnate or serrated. The leaf hair may be present on the lower side of the leaf, mainly on the nerve, on the whole leaf surface or be absent at all. The lower leaves are caudal, with a very wide top segment, while the side segments are triangular, with the apex facing the base of the leaf. The leaves of the stem are green with gray shades, oblong, lanceolate, with an arrow or heart shaped base**(Janda et al., 2021).**

**Figure 2:** the wild chicory plant (Cichorium intybus)**(Janda et al., 2021).**

### II.3.2 Taxonomic and chemical constituents

The name of the plant is derived from Greek and Latin. Cichorium means field and intybus is partly derived from the Greek “to cut”, because of the leaves, and partly from the Latin tubus to indicate the hollow stem. Its common names: Arabic: shikoryah, hidaba, hindaba bariah; Chinese: ju ju; English: Belgium endive, chicory, coffee chicory, French endive, succor, witloof; Franch: chicon, chicorée, chicorée à café, chicorée de, Bruxelles,chicorée sauvage, endive, endive witloof**(Al-Snafi, 2016)**

**Table 3**: Taxonomic Hierarchy of *Cichorium intybus***(Al-Snafi, 2016).**

| | |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Subclass | Asteridae |
| Order | Asterales |
| Family | Asteraceae |
| Genus | *Cichorium L.* |
| Specie | *Cichorium intybus* |

Chicory root contains some phytochemicals such as inulin (starch-like polysaccharide), coumarins, flavonoids, sesquiterpene lactones (lactucin and lactucopicrin), tannins, alkaloids, vitamins, minerals, and volatile oils. The very common phytocompounds are phenolic acids, which include chlorogenic acids, and flavonoids (anthocyanins, flavonols, flavanone, and flavan-3-ols). The plant polyphenols usually occur as glycosides, which makes them less reactive and easier to store in the cell vacuol. Cleavage of the glycosidic-linkage and associated rearrangement reactions releases the following sugar residues: hexose, glucose or galactose, deoxyhexose, rhamnose, pentose, xylose or arabinose, and glucuronic acid. Anthocyanins have been reported to reduce the risk of coronary heart disease in animals by exhibiting arterial protection, inhibition of platelet aggregation, and protection of endothelial tissues**(Nwafor et al.,2017).**

Sixty-four compounds were detected, which include several hydroxycinnamic acid derivatives comprising eight mono- and dicaffeoylquinic acids, three tartaric acid derivatives, thirty-one flavonol and two flavone glycosides, and ten anthocyanins as well as several isomers of caffeic acid derivatives**(Nwafor et al.,2017)**. The identification of some phytoconstituents of chicory as presented in **Figure 3.**

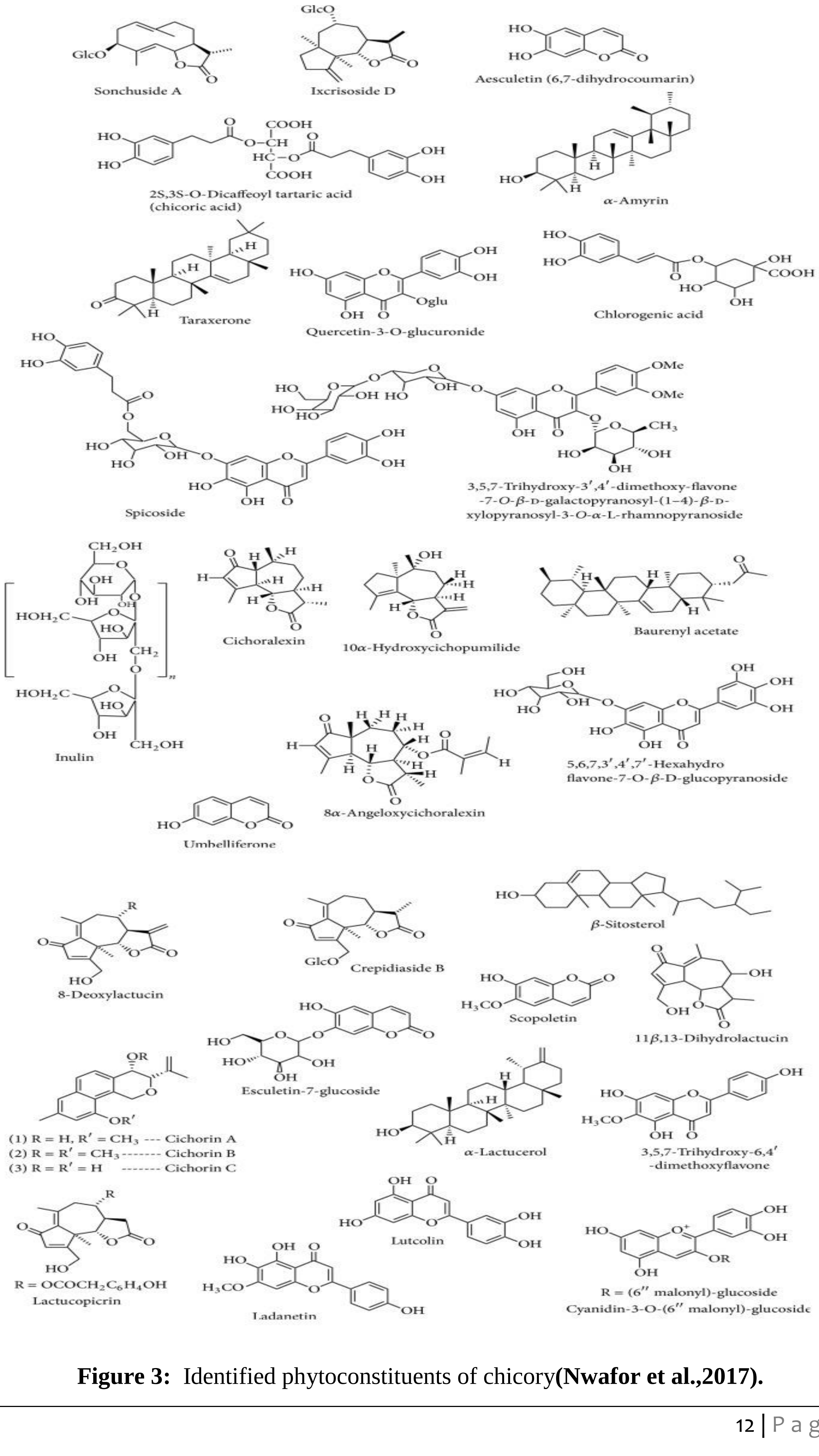



**Figure 3:** Identified phytoconstituents of chicory**(Nwafor et al.,2017).**

### II.3.3 Biological activities

The antibacterial activity of the organic acid-rich extract of fresh red chicory (*C. intybus* var. sylvestre) was tested against periodontopathic bacteria including *Streptococcus mutans, Actinomyces naeslundii,* and *Prevotella intermedia*. The compounds identified from the active extract include oxalic acid, succinic acid, quinic acid, and shikimic acid. All of the organic acids were found to decrease biofilm formation and adhesion of bacteria to the cells, with different levels of efficacy**(Street et al., 2013).**

The folkloric use of *C. intybus* as a hepatoprotectant has been well documented, a traditional Indian tonic used widely for hepatoprotection. In a randomized, double-blind clinical trial conducted on cirrhotic patients. The phenolic acid-rich seed extract of *C. intybus* was evaluated for its efficacy against hepatic steatosis *in vitro* and *in vivo*.The aqueous-methanolic extract of the seeds of *C. intybus* has been investigated for the hepatoprotective activity against acetaminophen and carbon tetrachloride-induced liver damage in mice**(Street et al., 2013).**

Chicory has reported antidiabetic activity. Based on the traditional use of *C. intybus* in diabetes mellitus, the hypoglycemic and hypolipidemic properties of the ethanol extract of the whole plant were investigated.Feeding the diabetic Wistar rats with *C. intybus* leaf powder led to a decrease in blood glucose levels to near normal value**(Street et al., 2013).**

The inhibition of TNF-*α* mediated cyclooxygenase (COX) induction by chicory root extracts was investigated in the human colon carcinoma (HT 29) cell line. The ethyl acetate extract inhibited the production of prostaglandin $E_2$ ($PGE_2$) in a dose-dependent manner. TNF-*α* mediated induction of COX-2 expression was also suppressed by the chicory extract**(Al-Snafi, 2016).**

Wound healing activity of the aerial parts, leaves, and roots as well as ashes of either leaves or roots were studied in rats. Subsequently, roots of the plant were submitted to further detailed investigations. The wound healing activity of the methanolic extract, its subextracts, and fractions were evaluated by using in vivo linear incision and circular wound models in rats. The hydroxyproline content of the tissues treated with extracts was also assessed for the activity evaluation. Moreover, in order to find out a possible involvement of antioxidant activity in wound healing, the test samples were also investigated by DPPH radical scavenging activity and total phenolic concentration were also determined**(Al-Snafi, 2016).**

## II.4 Silybum marianum

### II.4.1 Presentation of plant

Milk thistle (*Silybum marianum* L. Gaernt.), sometimes called wild artichoke, is a medicinal plant that has been used for thousands of years as a remedy for a variety of ailments. The milk thistle is an annual to biannual plant of the *Asteraceae* family, flowering in July–August with reddish-purple flowers. Milk thistle needs to grow in a warm atmosphere and dry soil and will grow up to 3 m high and 1 m across. However, it most commonly reaches 0.9–1.8 m in height **(Bijak, 2017).**

Native habitats of milk thistle are Southern Europe, Southern Russia, Asia Minor and Northern Africa, and is it naturalized in North and South America as well in South Australia. Milk thistle flower heads are 4–8 cm in diameter and contain around 50–200 tubular florets (individual flowers forming part of a group of flowers), which have a 13–25 mm dimension with color ranging from magenta to purple. The bracts below the flowers are broad and rigid with a rounded appendage ending in a spine. This plant has one long taproot. Milk thistle has variegated dark and light green spiny leaves with a length up to 75 cm and width up to 30 cm that are smooth on the upper surface and hairy on the lower surface. The leaves have milky-white veins, which inspired its common name of *Silybum marianum***(Bijak, 2017).**

There is confusion about whether milk thistle has fruits or seeds. Botanically correct, this plant has a cypselae, which looks like a seed but is technically a fruit. Each fruit (having a cocoa-like odor and an oily, bitter taste) is about 5–8 mm long, up to 2–3 mm wide, and 1.5 mm thick, with a glossy, brownish black to greyish husk. They are hairless but have a white, silky pappus (an appendage) of fine bristles. The fruits are joined together around the ring **(Figure 4).**

**Figure 4:** Milk thistle *(Silybum marianum L.)***(Bijak, 2017).**

### II.4.2 Taxonomic and chemical constituents

*Silybum marianum* (L.) a herbaceous plantbelonging to the carduae tribe of the Asteraceae(daisy) family. Its synonym is Cardus marianus L.The common name, milk thistle, is derived because ofthe white markings (variegation) on the leaves which,when break open, yield a milky sap**(Sidra Sabir et al., 2014).**

**Table 4:** Taxonomic Hierarchy of *Silybum marianum***(Sidra Sabir et al., 2014).**

| | |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Viridaeplantae |
| Infrakingdom | Streptophyta |
| Division | Tracheophyta |
| Subdivision | Spermatophyta |
| Infradivision | Angiospermae |
| Class | Magnoliopsida |
| Superorder | Asteranae |
| Order | Asterales |
| Family | Asteraceae |
| Genus | *Silybum* |
| Species | *Silybum marianum* |

Fruits of Silybum marianum(L.) Gaertn. Containsilymarin which is an isomeric mixture offlavonolignans. About 65 – 80% silymarin is present in its crudeextract of dried seeds. Silymarin is a complex of aboutseven flavonolignans such as silybin A and B,isosilybin A and B, isosilychristin, silydianin andsilychristin and the flavonoid taxifolin.AmongtheseSilybin, isosilybin, silychristin and silydianin areprincipal components of silymarin. The isosilybins and, Silybins are isomers. Silybin is alsoknown as silibin. It is regarded as the main activeingredient of milk thistle. Other flavonolignansidentified in milk thistle include dehydrosilybin,deoxysilydianin, deoxysilycistin, silybinome,silandrin, neosilyhermin and silyhermin. Beside theseapigenin; silybonol; myristic, oliec, palmitic andstearic acids; and betaine hydrochloride (which mayhave a hepatoprotective effect) are also identified in milk thistle.All the properties ofmilk thistle are due to these flavonolignans combined with other ingredients**(Sidra Sabir et al., 2014).**

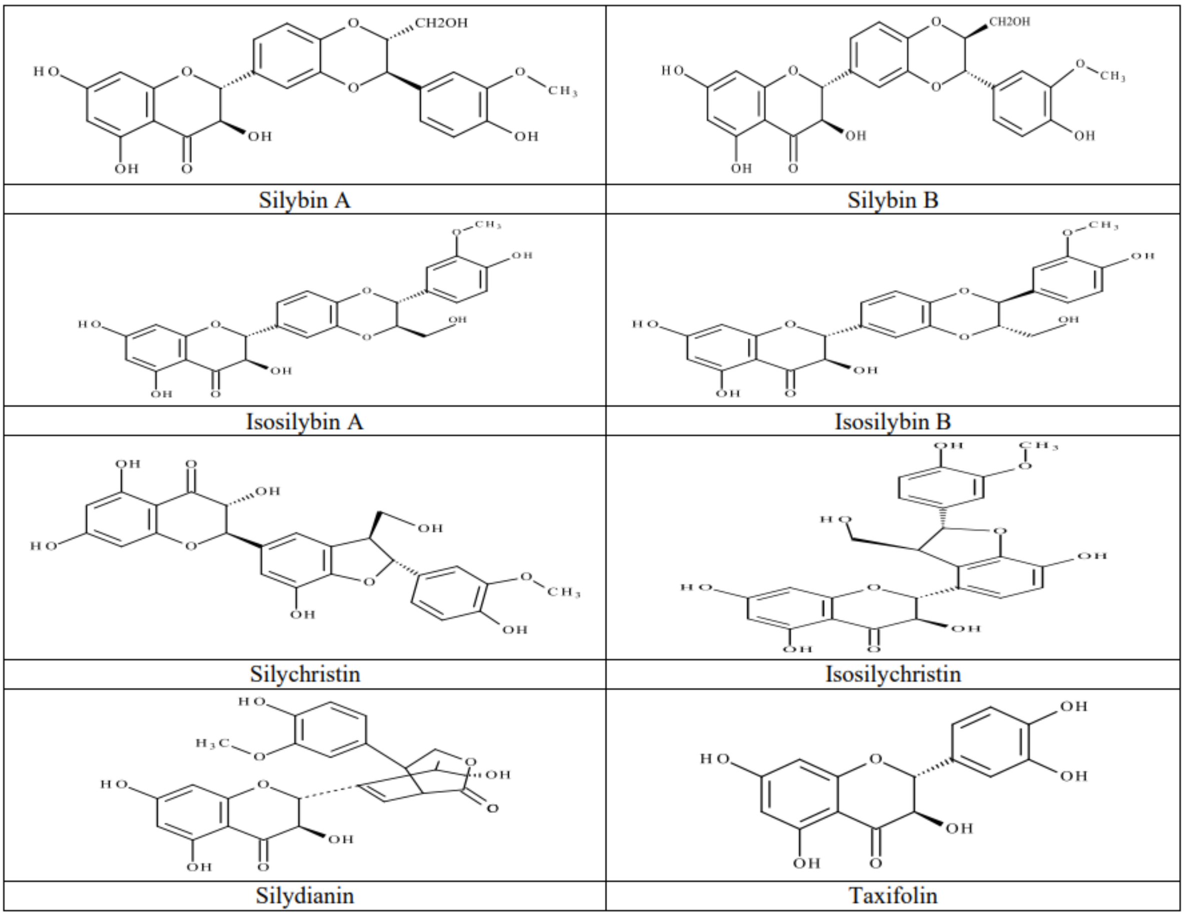



**Figure 5:** Chemical structures of several main silymarins in *Silybum marianum***(Le et al., 2018).**

### II.4.3 Biological activities

*Silymarin* inhibits the growth of *Candida albicans*, possibly via destruction of the membrane, and reduces its virulence through interference with the destabilization of mature biofilms and secretion of hydrolases. *Silymarin* reduces the viability of *Plasmodium falciparum* with a high selectivity index by inducing membrane damage and apoptosis via interaction with heme. *Leishmania* species are obligate intracellular parasites of mononuclear phagocytes transmitted by phlebotomine sandflies.Dehydroisosilybin exhibits antiproliferative effect against Leishmania promastigotes. Mayaro virus (MAYV) belongs to the Togaviridae family of positive, single-stranded, enveloped RNA viruses and causes Mayaro fever, which is characterized by fever, myalgia, headache, and diarrhea. Silymarin and silybin were found to inhibit the replication of MAYV and MAYV-induced oxidative stress in HepG2 cells.These results suggest that silymarin exerts antimicrobial effects via modulating both the microorganism and host responses, supporting further investigation of this compound as a novel therapeutic approach for infectious diseases in vitro, but it does not inhibit the multiplication of parasites at the intracellular stage**(Wang et al., 2020).**

To date, many studies have shown that SM possesses anticancer effects against various types of cancers, including gastric cancer, prostate cancer, hepatocarcinoma, laryngeal carcinoma, lung cancer, glioblastoma, breast cancer, multiple myeloma, glioma, colorectal cancer, malignant melanoma, bladder cancer, skin cancer, nasopharyngeal carcinoma, cervical cancer, and leukemiaSilybin meglumine impedes the epithelial to mesenchymal transition in non-small-cell lung carcinoma cells. Moreover, evidence supports the use of SM as an adjuvant chemotherapeutic drug to improve the anticancer effects and prevent the side effects of chemotherapy. For example, doxorubicin is a widely used anthracycline in cancer chemotherapy, and silymarin and its components protect cardiomyocytes against doxorubicin-induced oxidative stress, possibly via the stabilization of the cell membrane andelimination of free radicals and iron**(Wang et al., 2020).**

SM has been widely used for the treatment of liver diseases, including alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), and drug toxicity. Standard recommended doses of silymarin are safe and potentially effective in improving symptoms of acute clinical hepatitis. Many clinical and laboratory studies have documented that silymarin exhibits protective effects against stress-induced acute liver injury, liver fibrosis thalassemia, and acute hepatitis **(Le et al., 2018).**

The cardioprotective effect of SM has been documented not only in animal models of cardiovascular diseases but also in cardiovascular cell models. Inflammation, oxidative stress, and cell death are generally thought to be the main pathogenic mechanisms of cardiovascular disease, including heart failure, myocardial infarction (MI), and arrhythmia. During the acute phase of MI in pig models, SM increases myocardial salvage through preserving mitochondrial function via antioxidant effects. Moreover, SM improves cardiac healing and heart contractility post MI by decreasing cardiac reactive fibrosis in the border zone by inhibiting TGFβ1−TβR− Smad2/3 signaling pathways.The β-adrenergic agonist isoproterenol can cause cardiac myocyte injury, thereby leading to cardiac dysfunction**(Wang et al., 2020).**

Silybin intake ameliorated the impairment of learning and memory, SM may have the potential to prevent and treat neurological disorders, Parkinson’s disease, contribute to the amelioration of cerebral ischemia and cerebral stroke. Moreover, silymarin also exhibits protective effects against hair loss, acute radiodermatitis, skin explant deterioration, skin aging, and acne. Silymarin and silybin reduce blood glucose and plasma insulin levels in vivo.Treatment with silymarin protects against organ injury induced by manganese, copper, iron, and vanadium by promoting metal detoxification and antioxidant activity**(Le et al., 2018).**

# PART III. MATERIAL AND METHODES

## III.1 Objective of study

Our study was carried out in the biology pedagogical laboratory of the University of Saida-Dr. Moulay Tahar, whose main objective is to study the antioxidant activity of the extract of three plants (AP, CI and SM) and their combination. The phytochemical screening of each plant was determined, and the antioxidant activity was investigated by using reduced power, 1,1-diphenyl-2-picrylhydrazyl (DPPH). The diagram below briefly explains the experiment:

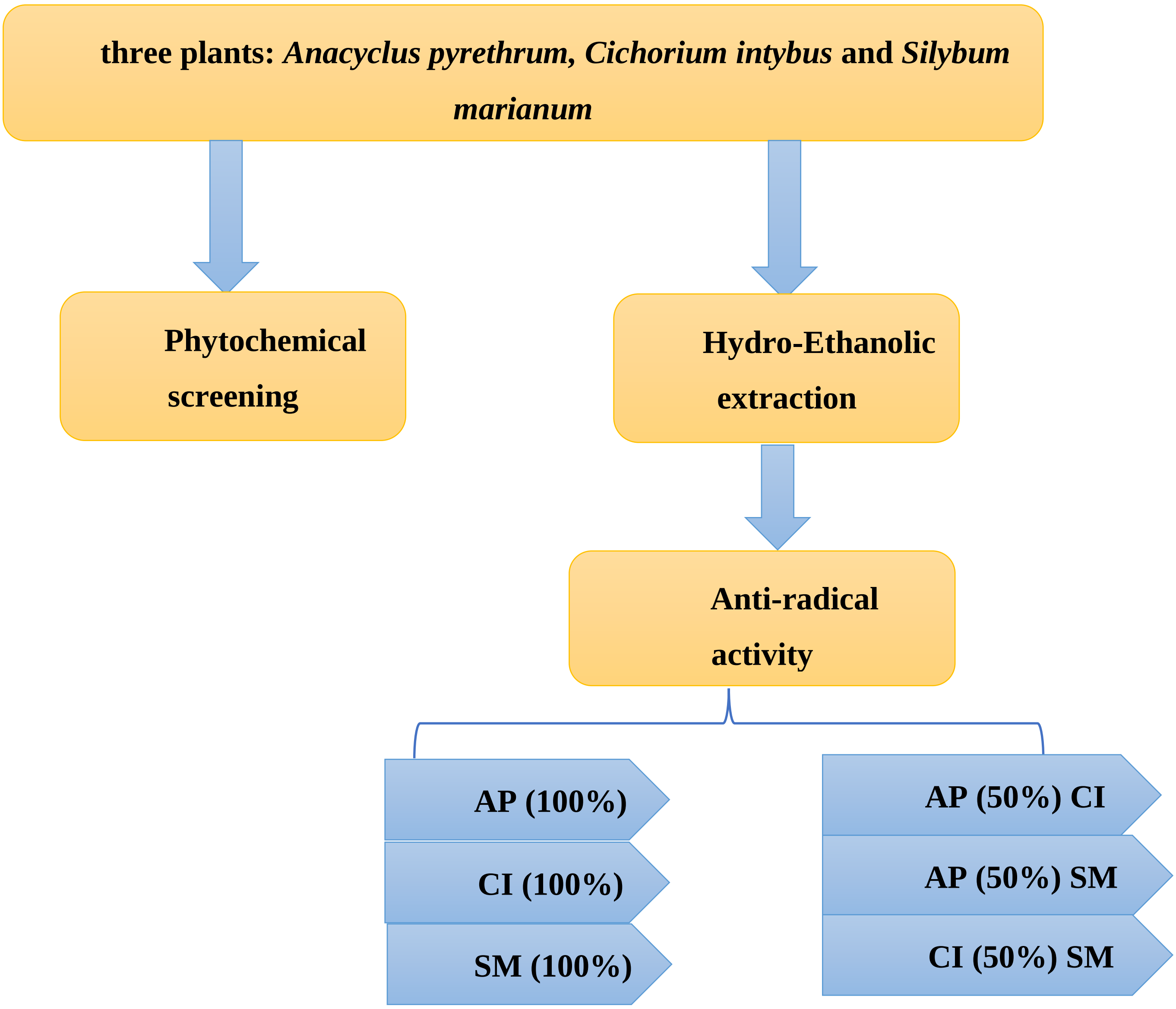



**Figure 6:** General diagram of the analyses carried out

### III.2 Sampling

The roots of *Anacyclus pyrethrum*, stems of *Cichorium intybus*, as well as the seeds of *Silybum marianum*, were purchased from a herbalist in the Saida region. The plants are rinsed with water and dried.

***Anacyclus pyrethrum*** (roots) ***Cichorium intybus*** **(stem)** ***Silybum marianum*** **(seeds)**

**Figure 7:** photos of plants samples

### III.3 Extract Preparation

The collected samples ground to a fine powder using an electric blender, then 10 g of the dried powder was extracted and was extracted by maceration with 1000 mL of 70% ethanol at room temperature for 48 h. Extracted contents were filtered and concentrated on a rotary evaporator to reduce the volume. On complete drying of the solvent using a fume cupboard for 24 h; the obtained yield was measured by applying Equation below. The samples obtained were stored at 4 °C in glass stopper vials to study their antioxidant activity **(Javeed et al 2022; Jawhari et al.,2020).**

**Yield (%) =**(Weight of the extract (g) / Weight of the dried sample (g)) * 100%

### III.4 Phytochemical Screening

As part of the research on the bioactive molecules present in our plants which are responsible for biological effects, we carried out a qualitative study called phytochemical screening, it is a process that analyzes and identifies the chemical compounds, including secondary metabolites, present in plants based on solubility tests, on coloring and precipitation reactions as well as on examinations in ultraviolet light.

### III.4.1 Tannins

The presence of tannins is demonstrated by adding 2 ml of water and 2 to 3 drops of diluted $FeCl_3$ solution (1%) to 1 ml of ethanolic extract. The appearance of a blue-black color characterizes the presence of gallic tannins, and a green or blue-green color characterizes the presence of catechol tannins **(Aiyegoro and Okoh, 2010).**

### III.4.2 Flavonoids

The flavonoid detection reaction consists of treating 5 ml of ethanolic extract with 1 ml of concentrated HCl and 0.5 g of magnesium turnings. The presence of flavonoids is demonstrated if a pink or red color develops after 3 minutes**(Siddiqui et al., 2009).**

### III.4.3 Anthocyanins

A volume of 2 ml of infusion is added to 2 ml of 2N HCl. The appearance of a pink-red color that turns blue purple upon the addition of ammonia indicates the presence of anthocyanins**(Siddiqui et al., 2009).**

### III.4.4 Coumarins

A 1-gram mass of plant powder is placed in a tube with a few drops of distilled water. The tubes are covered with paper soaked in diluted NaOH and brought to a boil. Any yellow fluorescence indicates the presence of coumarins after examination under ultraviolet **(Aiyegoro and Okoh, 2010).**

### III.4.5 Alkaloids

We performed a 24-hour maceration of 2 grams of plant powder mixed with 50 ml of half-strength $H_2SO_4$ and distilled water. We filtered the mixture and rinsed it with water to obtain 50 ml of filtration. We then took two test tubes into which we added 1 ml of maceration. We added 5 drops of Mayer's reagent to tube 1 and 5 drops of Wagner's reagent to tube 2. The presence of turbidity or a precipitate after 15 minutes indicates the presence of alkaloids**(Siddiqui and Ali., 1997).**

### III.4.6 Saponins

Their presence is determined quantitatively by calculating the foam index, the degree of dilution of an aqueous decoction that produces a persistent foam under specific conditions. We prepared a decoction of 2 grams of plant powder with 100 ml of distilled water, which was brought to a boil for 30 minutes. After cooling and filtering, the volume was adjusted to 100 ml. From this stock solution, 10 tubes (1.3 cm internal diameter) were prepared, with 1.2 to 10 ml, the final volume being adjusted to 10 ml with distilled water. Each of these tubes was shaken vigorously in a horizontal position for 15 seconds.

After standing for 15 minutes in a vertical position, the height of the persistent foam was recorded in centimeters. If it was close to 1 cm in the 10th tube, then the foam index was calculated using the following formula:

I = Foam height (in cm) in the 10th tube x 5 / 0.0 X

X: This is the height of the foam.

The presence of saponins in the plant is confirmed with an index greater than 100**(Siddiqui and Ali., 1997).**

### III.4.7 Reducing Compounds

Their detection consists of treating 1 ml of the ethanolic extract with distilled water and 20 drops of Fehling's solution, then heating. A positive test is revealed by the formation of a brick-red precipitate **(Kholkhal, 2014).**

### III.4.8 Starch

Heat 5 ml of the aqueous extract with 10 ml of saturated NaOH solution in a water bath until boiling. Then add the starch reagent. A positive test is revealed by the appearance of a blue-purple color **(Kholkhal, 2014).**

## III.5 DPPH Scavenging activity

### III.5.1 Principe

One of the first free radicals used to study the structure-antioxidant activity relationship of phenolic compounds is the chemical compound 2,2-diphenyl-1 picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl). It has an unpaired electron on an atom of the nitrogen bridge, this delocalization causes the appearance of the characteristic violet color of the DPPH• solution **(Figure 8).** Measurement of the effectiveness of an antioxidant is done by measuring the decrease in the violet color, due to a recombination of the DPPH• radicals **(Cristina et al., 2009).**

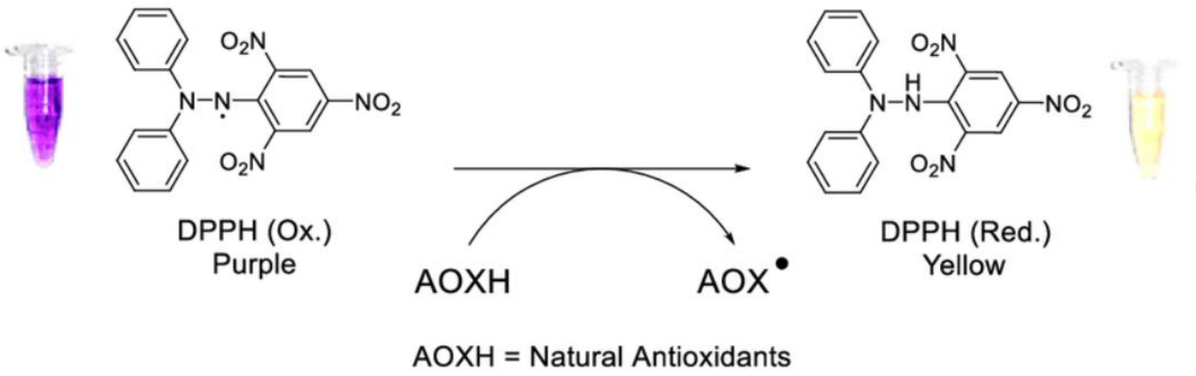



**Figure 8:** DPPH reaction with natural antioxidants**(Arce-Amezquita et al., 2019).**

### III.5.2 Dosage

The stock solution was prepared by dissolving 2,4 mg DPPH on 100 ml of ethanol. The test is performed by mixing a volume of 2,7ml of the previous solution of DPPH with 300 µl of extracts of our samples or standard antioxidant (ascorbic acid) at different concentrations (0 - 1000 µg/ml). After 30 min of incubation in the darkness and at room temperature, the absorbance is read at 517 nm using the spectrophotometer against a blank that contains only ethanol. The percentage of inhibition was calculated using the formula:

%Inhibition(mg/mL) = [(A_control - A_sample)/A_control]* 100

where A_control is the absorbance of the control and A_sample is the absorbance of the test compound.

The sample concentration that induces 50% inhibition (IC50) was determined from agraph plotting the percentages of inhibition against the sample concentrations **(Baslam et al., 2023; Elazzouzi et al., 2019)**

### III.6 Preparation of combination and statistical analysis

In the present work, extracts were studied separately or in combination in a specific ratio in ethanol. Extract plants samples were studied at concentration of 1mg/ml: one mg of each sample of extract was treated with 1 ml of ethanol. For combination, 50:50 ratio was prepared 0,5mg of the first extract plant was mixed by 0,5mg of the second extract plant and treated with 1 ml of ethanol. The results obtained are processed by Excel.

# PART IV. RESULTS AND DISCUSSION

## IV.1 Extraction Yield

The yield (appearance and color) of each extract is represented in **Table 5.** The results showed that the hydro-ethanol extracts of different parts of plants varieties have two aspects: pasty and powdery with two different colors (green and brown). The maximum yield was obtained from SM seeds extracts (16%), followed by the AP roots extracts (10%), while the CI stems presented the lowest yield (5,75%).

**Table 5:** Aspects, colors, and yields of extracts from the three plants.

| **Plants (parts)** | **Aspects** | **colors** | **Yield** |
|---|---|---|---|
| *Anacyclus pyrethrum (roots)* | Pasty | Brown | 10% |
| *Cichorium intybus(*stems) | Powder | Green | 5,75% |
| *Silybum marianum* (seeds) | Powder | Brown | 16% |

Our results are similar to those found by**Jawhari et al., (2021; 2020)**, who reported that the AP extract was patsy, brown and the yield was 10%. Regarding the CI result of **Kaçmaz et al., (2025),** found that the yield of methanolic extraction of the CI stem was a litter higher with 7.02%, our SM result was similar to the study done by **Lukic et al., (2022),**they reported a value of 17.2%. On the contrary the extraction of the SM seeds was 5.01% in the research of **Javeed et al., (2022).**

## IV.2 Qualitative Phytochemical Analysis

The analysis of the phytochemicals qualitatively revealed the existence of alkaloids in the three plants, flavonoids and catcholic tannins in CI and SM. in addition we found the anthocyanins and Starch in CI and SM extracts respectively. However, Coumarins, Reducing Compounds, Saponins and gallic tannins were absent in all plants. The results for phytochemicals are represented in **(Table 6).**

**Table 6:** Qualitative analysis of phytochemicals from different plants

| **Phytoconstituents** | **Plants** | | |
|---|---|---|---|
| | ***Anacyclus pyrethrum*** | ***Cichorium intybus*** | ***Silybum marianum*** |
| **Alkaloids** | + | + | + |
| **Anthocyanins** | - | + | - |
| **Coumarins** | - | - | - |
| **Flavonoids** | - | + | + |
| **Reducing Compounds** | - | - | - |
| **Saponins** | - | - | - |
| **Starch** | - | - | + |
| **Tannins (Catcholic)** | - | + | + |
| **Tannins (Gallic)** | - | - | - |

+ (presence of phytoconstituents); - (absence of phytoconstituents).

Alkaloids play an essential role in both human medicine and in an organism's natural defence. Alkaloids make up approximately 20% of the known secondary metabolites found in plants. In plants, alkaloids protect plants from predators and regulate their growth. Therapeutically, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents. Well-known alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine**(Heinrich et al., 2021).**Alkaloids have been shown to have substantial antioxidant activity, suggesting that these natural-product-inspired bioactive entities may have major beneficial influence on human health and food processing sector**(Atpadkar et al., 2023).**

The anthocyanins, which are the most important group of flavonoids in plants, are pigments with a flavylium cation (AH+) structure that act as acid. This structure is directly related to its antioxidant activity. Most of the functional properties and the sensory quality of the anthocyanins can be explained by their chemical reactivity. The structures and properties of anthocyanins are dependent on different factors such as pH, temperature, and solvents which should be controlled to carry out antioxidant activity studies of these compounds**(Tena et al., 2020).**

Coumarins consisting of fused benzene and α-pyrone rings are present in significant amounts in plants, and more than 1300 coumarins have been identified from natural sources. Derivatives of coumarins naturally occur as secondary metabolites present in seeds, roots, and leaves of many plant species. Coumarins have a variety of important biological activities such as anti-inflammatory, antioxidant, antiviral, antimicrobialand anti-cancer. Coumarins are indicated to increase central nervous system activity**(Al-Majedy et al., 2016).**

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure. They have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions**(Panche et al., 2016).**

Reducing compounds, often called antioxidants, are substances that can donate electrons or hydrogen atoms to free radicals, neutralizing them and preventing oxidative damage. They are crucial for protecting cells and tissues from the harmful effects of ROS**(Ullah et al., 2020).**

Saponins are glycosides of triterpenes and steroids. The highly polar sugar moieties together with the non-polar triterpene or sterol backbones result in a highly amphipathic compound**(Mugford and Osbourn, 2013).**Saponins possess antioxidant activity, helping to neutralize free radicals and oxidative stress in the body**(Timilsena et al., 2023).**

tannins are water-soluble phenolic compounds with molecular weights in the range of 300 to 500 and could precipitate gelatin, alkaloids, and proteins. Tannins are secondary metabolites widely distributed in plants: they are polymeric phenolic substances. In general, tannins can be classified into one of two classes based on their structural features: hydrolyzable and condensed tannins**(Ojo, 2022).**Due to their high polyphenolic content, tannins are considered potent antioxidants, effectively scavenging free radicals, reducing oxidative stress**(Cosme et al., 2025).**

The results **Baslam et al., (2023)** revealed the presence of total polyphenols, flavonoid, and condensed tannin in AP plant. Also, **Abbaset al., (2015)** found that CI plants contain Tannins, flavonoids and saponins. The analysis of the phytochemicals qualitatively found in the study of **Javeed et al., (2022).** revealed the existence of various phytoconstituents such as alkaloids, glycosides, flavonoids, terpenoids, steroids and catcholic tannins in the seeds of SM except for saponins and Gallic tannins.

## IV.3 Evaluation of anti-radical activity by DPPH

### IV.3.1 Individual plant

Recently, antioxidants have become a topic of increasing interest, not only to health and food science researchers and medical experts, but also to the public. Unfortunately, a validated method is still undeveloped for the quantification of antioxidant activity in food and biological samples **(Tlilani and Bensaida, 2019).** In this present work, the antioxidant activity of three plants extracts was evaluated in vitro by DPPH test. Due to its stability in radical form and the simplicity of analysis, the DPPH° radical is generally considered one of the most used compounds for the rapid and direct evaluation of antioxidant activity **(Bozin et al., 2008).** The chemical compound 2,2-diphenyl-1 picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl) was used for the detection of the anti-radical activity of three extracts plants: AP, CI and SM, as well as the mixture between them. Antioxidant molecules can neutralize DPPH• free radicals and convert them into other more stable compounds, which induces a decrease in the violet coloration. The IC50 value is inversely related to the antioxidant capacity of a compound, since it expresses the amount of antioxidant needed to decrease 50% of the radical concentration and therefore, the lower the IC50 value, the greater the antioxidant activity **(Villaño et al., 2007).**

**Figure 9** represents the percentage of inhibition of ascorbic acid used as a standard. The % inhibition varies from 2% to 71.42% depending on the concentration varies from 3.9 µg/ml to 1000 µg/ml respectively.

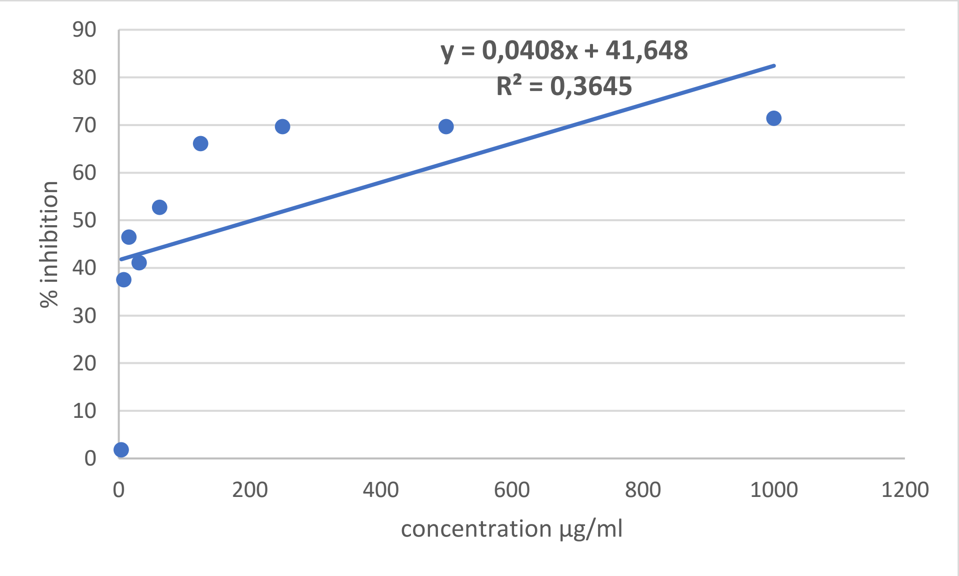



**Figure 9:**Inhibition percentage of ascorbic acid

Ascorbic acid, commonly known as vitamin C, is one of the basic and best-known compounds necessary for the proper functioning of the human body **(Gęgotek and Skrzydlewska, 2023).** Ascorbic acid is one of the basic low-molecular antioxidants functioning in the human body. It takes part in the regulation of the levels of ROS and the effectiveness of other antioxidants. Ascorbic acid regulates the level of ROS as early as at the stage of their formation **(Gęgotek and Skrzydlewska, 2022).**

**The figures 10, 11** and **12** show the antioxidant activity results of the three plants AP, CI, and SM, respectively. The percentage of AP inhibition ranges from 19.09% to 77.27% depending on the concentration of 3.9-1000 µg/ml, although the lowest % inhibition of CI is 7.27% and the highest is 70.91%. While the SM plant extract has an inhibition % varying between 3.63% and 33.63%.

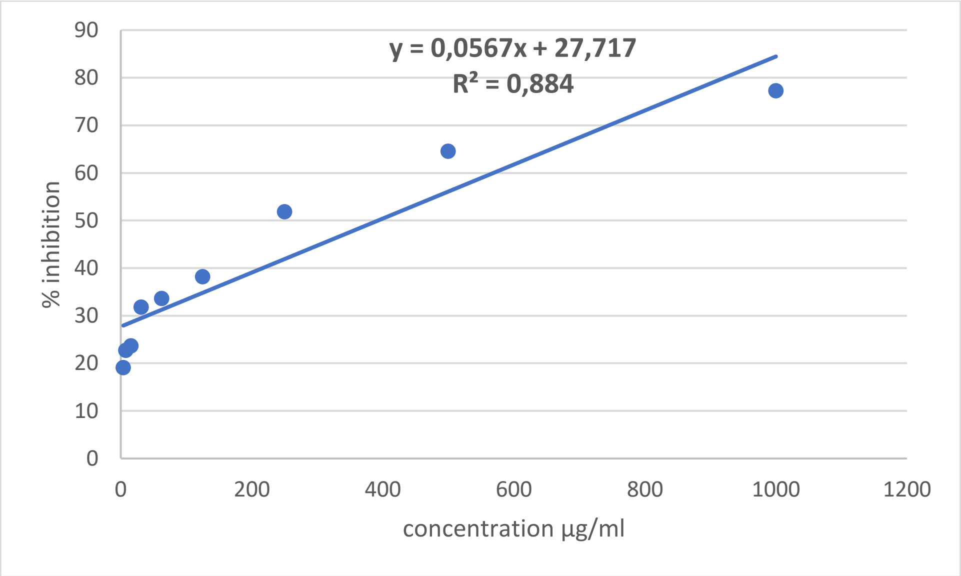



**Figure 10:** Inhibition percentage of *Anacyclus pyrethrum* (AP)

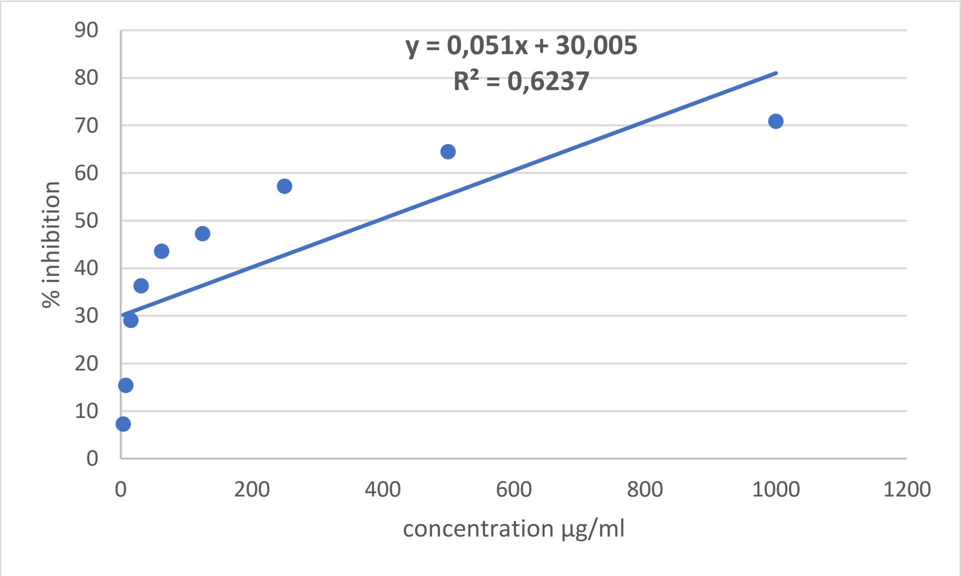



**Figure 11:** Inhibition percentage of *Cichorium intybus* (CI)

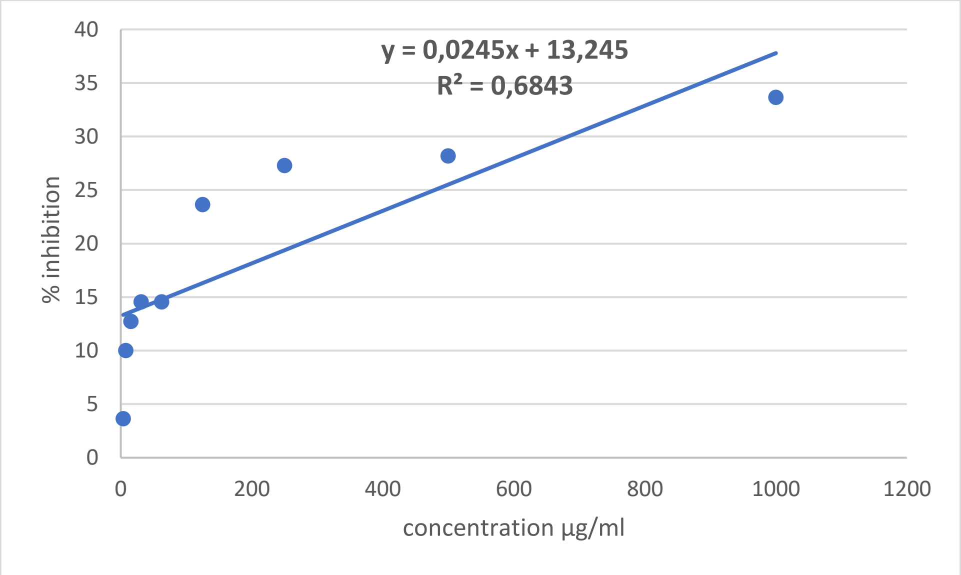



**Figure 12:** Inhibition percentage of *Silybum marianum* (SM)

The figure below represents the differences in inhibition % between ascorbic acid and the three plant extracts. We noticed that ascorbic acid has the highest inhibition % in most concentrations; thus the results show that AP represents the highest % in the concentration 1000 µg/ml with inhibition % 77.27% compared to ascorbic acid, CI and SM, but the CI plant extract has a high inhibition % in the concentrations of 15.6 µg/ml up to 500 µg/ml with inhibition % of 29.09% to 64.54% respectively, this means that CI has a significant antioxidant activity than AP and SM. On the other hand, we noticed that SM has the lowest antioxidant activity.

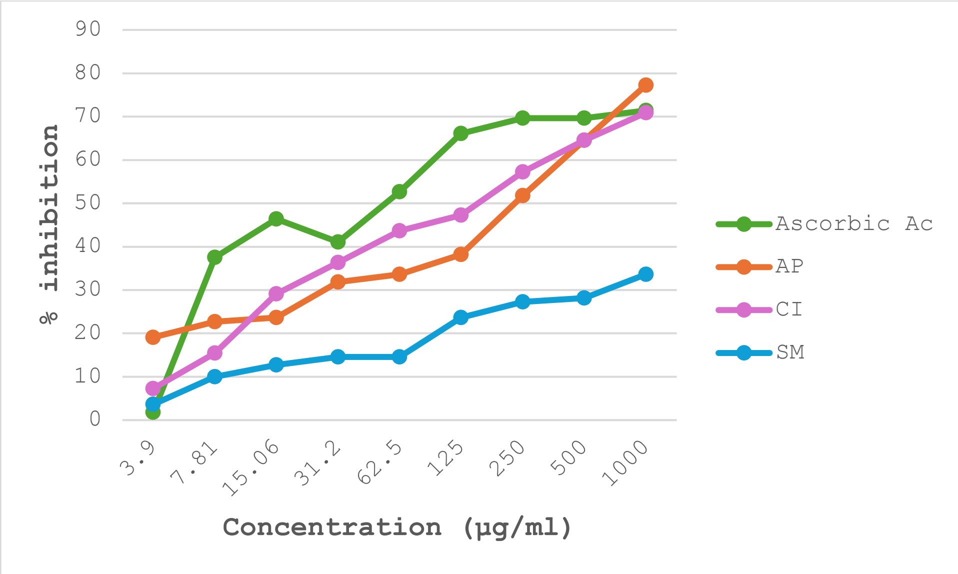



**Figure 13:** Comparison of the percentage of inhibition between ascorbic acid and the three plant extracts

Several studies on the chemical composition of AP have been previously published, confirming the presence of a wide variety of phytochemicals, of which about one hundred different compounds have been described to date like pelletonin, tannin, gum, potassium sulfate, carbonate, potassium chloride, and calcium phosphate. As reported in earlier data, the Ap possesses interesting pharmacological properties including anticancer, aphrodisiac, anticonvulsive, androgenic and fertilizing, antiparasitic and antibiotic, bioinsecticide, antidiabetic, antifungal and immunostimulant effects **(Elazzouzi et al., 2022; Mohammadi et al., 2017).**

All parts of CI possess great medicinal importance due to the presence of a number of medicinally important compoundssuch as alkaloids, inulin, is well known for their richness in phenolic compounds with antioxidant properties, namely, coumarins such as aesculetin, esculin, chicoriin A-B-C, scopoletin and 6,7-dihydroxycoumarin , hydroxycinnamic acids, vitamins, phenolic acids (caffeic and chlorogenic acid derivatives such as chicoric acid and neochlorogenic acid), flavonoids, and sesquiterpenes lactones . P. coronopus, also known as buck's-horn plantain, is mainly known for their content in phenylpropanoids such as verbascoside and plantamajoside **(Ceccanti et al., 2022 ; Abbas et al., 2015).** CI has been traditionally used for the treatment of fever, diarrhoea, jaundice and gallstones. The studies on rats have shown that CI has anti-diabetic activities. It has been also reported that C. intybus possesses anti-bacterial, anti-inflammatory, hyperglycaemic and anti-ulcerogenic activities

**(Abbas et al., 2015).** Among these medicinal plants, has received special attention for the treatment of gastrointestinal and liver problems **(Moloudi et al., 2021).** Moreover, it is used for their high radical scavenging and cytotoxic activity **(Piccolella et al., 2024; Ceccanti et al., 2022)**

SM has been used in traditional medicine since ancient times mostly for treatment of liver disorders and protection of the liver from toxins It hasbeen also reported that the S. marianum extracts show numerous beneficial pharmacological effects such as anti-inflammatory, antioxidant, nephron- and cardiovascular protective, anti-cancer and neuroprotective. The extracts from the S. marianum seeds can slow down the aging of the skin by radical-inhibiting activity and can reduceUVA-induced skin damage. The seeds contain a high amount of oil rich in unsaturated fatty acids such as linoleic (polyunsaturated omega-6 essential fatty acid) and oleic (monounsaturated omega-9 fatty acid) **(Lukic et al.,2022).** Recently, milk thistle has gained more attention due to its antihyperglycemic properties (**Qin et al., 2017).**

### IV.3.2 Binary mixtures

**Figures 14, 15**and **16** represent the % inhibition of the three mixtures, while the AP+CI mixture shows a percentage of inhibition varying between 11.81% at low concentration and 60.09% at high concentration, the % inhibition of AP+SM is between 5.45% and 64.54% in the concentrations 3.9 and 1000 µg/ml respectively, regarding the results of % inhibition of CI+SM mixture represents 13.63% as a % inhibition of the low concentration and 60.90% as a % inhibition of the highest concentration. From these results we notice that the % inhibition of the three mixtures are close and 64.54% represents the highest % inhibition.

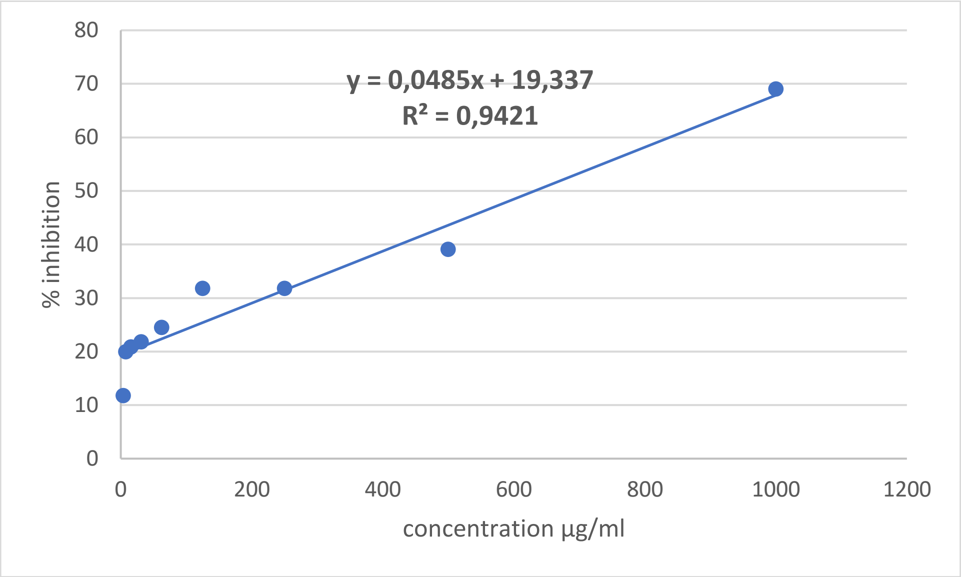



**Figure 14:** Inhibition percentage of AP+CI

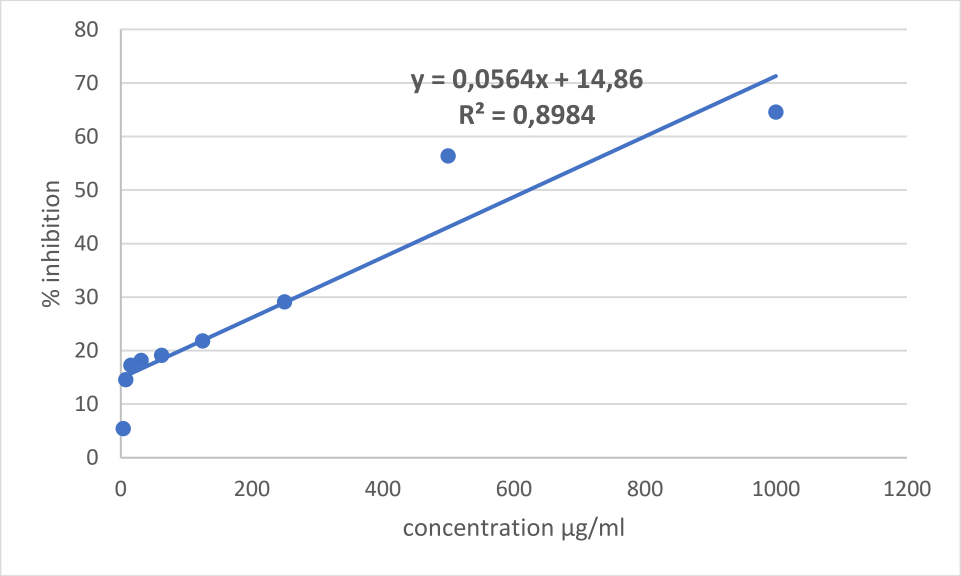



**Figure 15:** Inhibition percentage of AP+SM

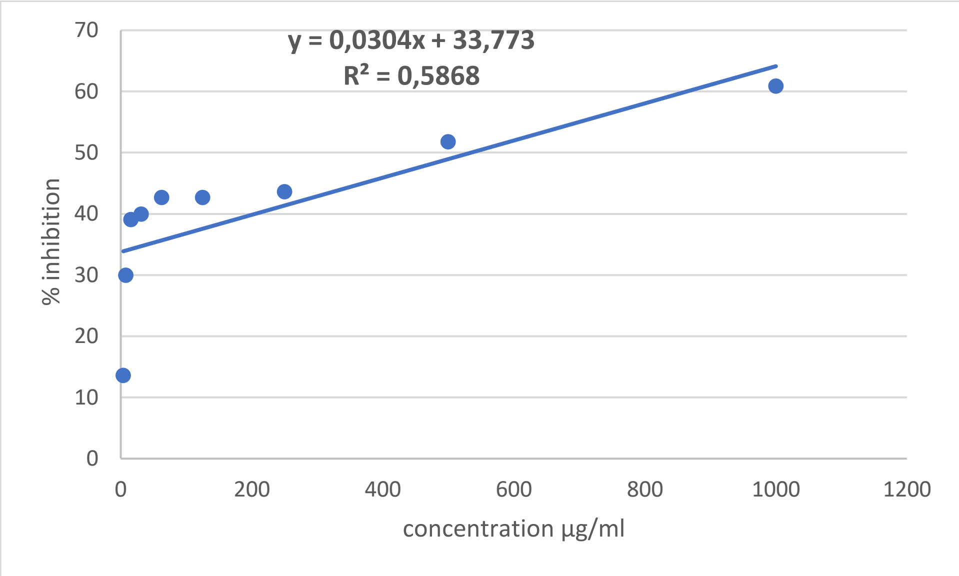



**Figure 16:** Inhibition percentage of CI+SM

In the figure below shows the results of the comparison of % inhibition of the plant extract AP and CI with their mixture (AP+CI), we notice that the % inhibition of AP+CI is low compared to AP and CI individually in the different concentrations; which means that the extract of the plants AP and CI each inhibits the antioxidant effect of the other, an antagonistic effect.

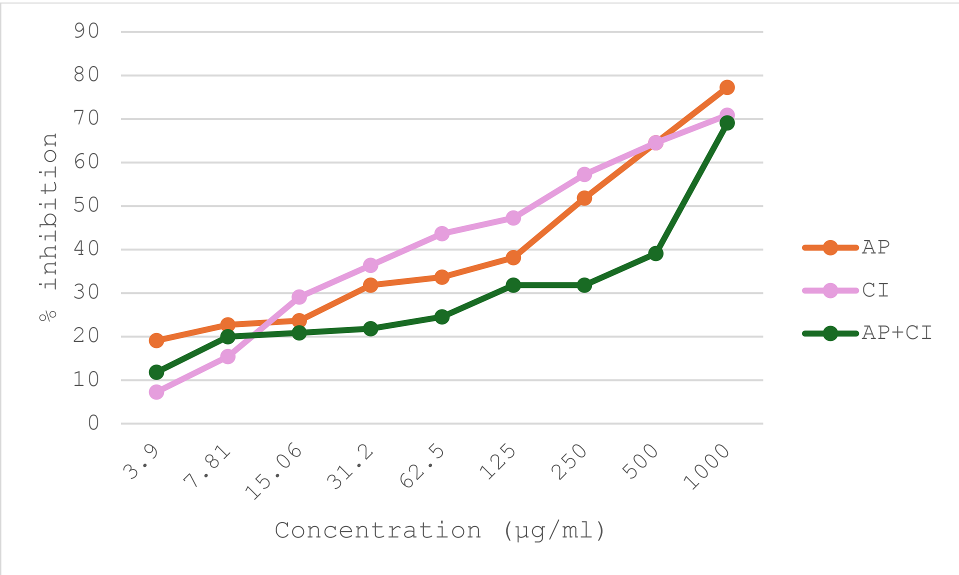



**Figure 17:** Comparison of the percentage of inhibition between AP, CI and their mixture

**Figure 18** shows the difference in % inhibition between AP and SM plant extracts compared to their mixture (AP+SM). The graphical results clarify that the % inhibition of AP+SM in the different concentrations is between the % inhibition of AP (higher) and SM (lower), this shows that the antioxidant effect of AP extract increases the antiradical effect of SM extract, which gives a synergism effect.

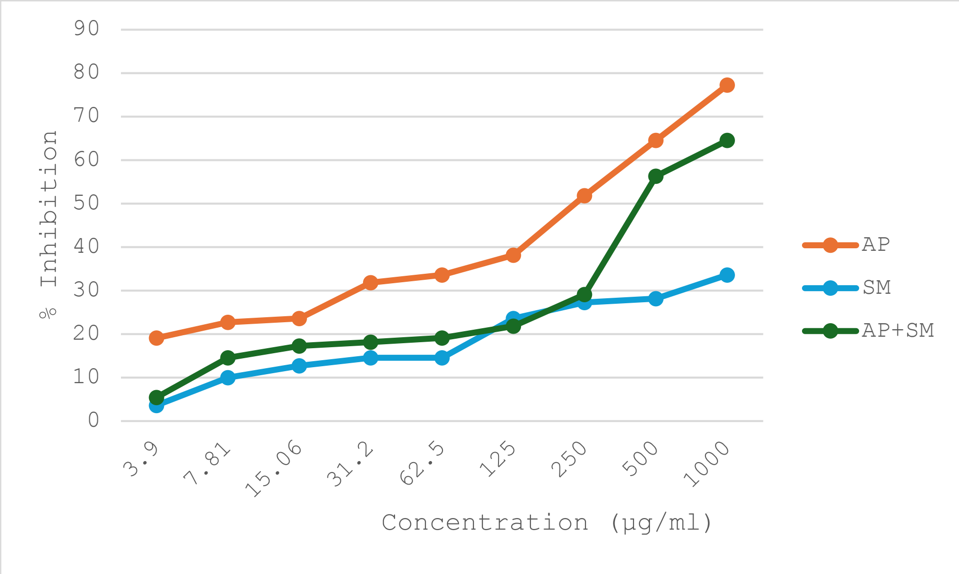



**Figure 18:** Comparison of the percentage of inhibition between AP, SM and their mixture

The figure below represents the difference in % inhibition between the extracts of the CI and SM plants compared to their mixture (CI + SM), our results indicate that the percentage inhibition of the CI + SM mixture is higher than the % inhibition of individual SM in all concentrations, while there is an increase in % inhibition of CI + SM compared to individual CI in the four concentrations from 3.9 to 31.2 µg / ml, a similarity in the concentration 62.5 µg / ml with % inhibition 43%, then a decrease in % inhibition of the mixture compared to individual CI in the four higher concentrations from 125 up to 1000 µg / ml. It can be said generally that the addition of the CI plant extract strongly increases the antioxidant effect of the SM plant extract, synergism effect.

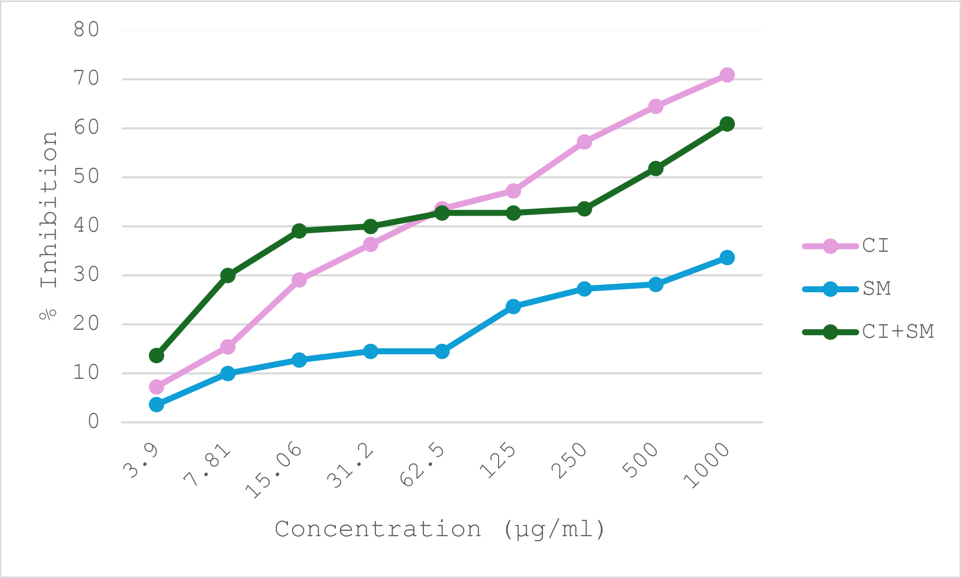



**Figure 19:** Comparison of the percentage of inhibition between CI, SM and their mixture

One of the observed antioxidant effects in a complex mixture is synergism. In other words, synergism is a coordinated or correlated action of two or more structures, agents, or physiological processes so that the combined action is greater than the sum of each acting separately. The synergistic antioxidant effect can be achieved also by the protection action of one antioxidant by means of its sacrificial oxidation. The mechanism responsible for synergistic antioxidant activity has not been explained yet mainly due to the complex nature of the mixture especially plant extracts. The synergism can be explained, formation of stable intermolecular complexes between the antioxidant's mixture, exhibit higher antioxidant activity than that of the parent compounds, formation of dimers and adducts or/and new phenolic products with a greater antioxidant power than that of the parent's compounds mixture and unpredictable interactions between the examined compounds **(Olszowy-Tomczyk, 2020).**

Sometimes antioxidants action in extract plants mixture can cause dramatic consequences because its antioxidant value can be considerably reduced. several hypotheses have been proposed to explain the mechanism of antagonistic antioxidant effect in a complex mixture such as possibility of complex and adduct formation between antioxidants, possibility of antioxidants polymerization which causes the decrease of their antioxidant properties, irreversible reactions of free antioxidant radicals leading to its final disappearance (hence they do not react with the radical which is neutralized)and undefined mutual interactions between antioxidants **(Olszowy-Tomczyk, 2020).**

### IV.3.3 Inhibitory concentration 50

The figurebelow represents the inhibitory concentration 50 of the extracts of the three plants and their mixtures, the results show that IC50 of AP and CI (393 and 392 µg/ml respectively) is higher than that of ascorbic acid with IC50 is 204.7 µg/ml. IC50 of SM is 1500.2 µg/ml which means that the SM extract has low antioxidant activity. However, the results of IC50 show that the extract of AP and CI increases the antiradical effect of SM with IC50 of AP+SM and CI+SM are 623.05 µg/ml and 533.78 µg/ml respectively. While the mixture of the extract AP and CI causes a decrease in antioxidant activity compared to the two individual plants with IC50 is 632.22 µg/ml.

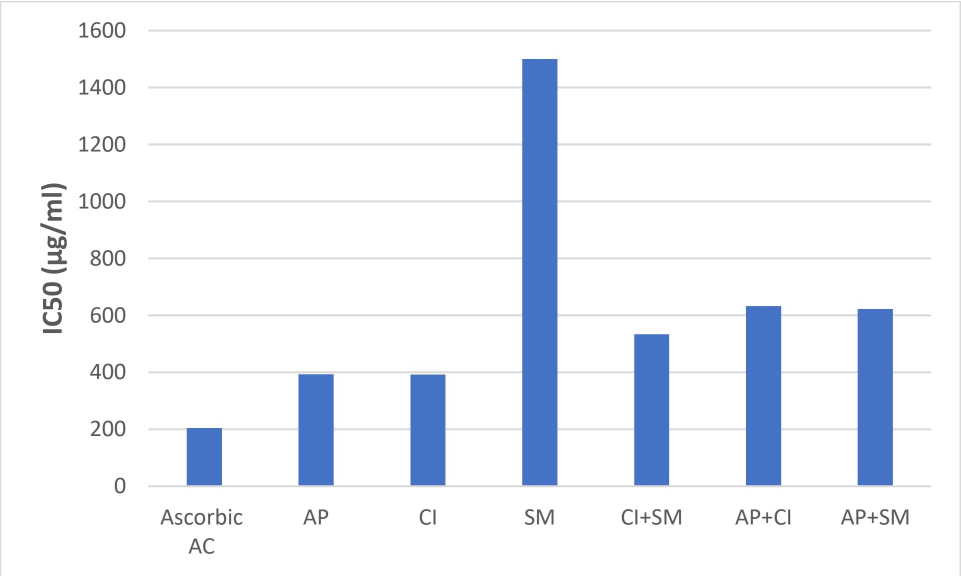



**Figure 20:** inhibitory concentration 50 of extracts plants and their mixtures

Our results show that the IC50 of AP extract is 393 µg/ml, however other studies have reported that AP extracts have lower IC50 value than our results, **Elazzouzi et al., (2019)** found that the IC50 of AP extract was 152 µg/ml, also **Jawhari et al., (2021) and Manouze et al., (2017)** showed that the IC50 of AP extract was 180 µg/ml and 13.41 µg/ml respectively. In addition, methanolic extracts of the aerial parts of A. pyrethrum reported by **Selles et al., (2012)**had a high ability to scavenge free radicals given their IC50 of 56 µg/ml. In contrast, **Baslam et al. (2023)** observed a high IC50 value for PA extract, which was 1600 µg/ml. Regarding our IC50 value for CI extract, it was 392 µg/ml. These results are consistent with those reported in a study by **Matvieieva et al. (2023),** who investigated the IC50 value of CI ethanol extracts, which was 346 µg/ml. **Saggu et al. (2014)** showed that the IC50 value of chicory extract was 100 µg/ml. In addition, the IC50 value of chicory leaf extract was 67.2

µg/ml **(Abbas et al., 2015).** Our finding on the IC50 value of SM extract was 1500 µg/ml, unlike our results **Javeed et al., (2022) and Serçe et al., (2016)** found that SM extract had good antioxidant activity with IC50 of 75.98 µg/ml and 92 µg/ml respectively.

There was a relative dependence between the antioxidant activity and the contents of the total polyphenols and flavonoids. For example, the extract which represents the high fraction of polyphenols and flavonoids, has a greater trapping effect than the other extracts. If we compare the contents of total phenols and flavonoids in crude and butanolic extracts, it appears that the extract, which is richer in polyphenols or flavonoids, has a lower antioxidant power. This can be explained by the presence of metabolites in the crude extract that augmente antioxidant activity. In fact, the phenolic fraction does not incorporate all the antioxidants and the synergistic interactions between the antioxidants in a mixture makes that the antioxidant activity depends not only on the concentration (quantitative), but also on the structure and nature of the antioxidants (qualitative) **(Elazzouzi et al., 2019).**

# PART V. CONCLUSION AND PERSPECTIVES

## Conclusion and perspectives

The medicinal plants possess potent antioxidant properties due to the presence of various bioactive compounds like polyphenols and flavonoids. These antioxidants help protect cells against damage caused by free radicals and oxidative stress, potentially reducing the risk of chronic diseases.

The objective of this work is to compare the antioxidant effects of three plants AP, CI and SM, as well as their binary mixtures using the DPPH test.The present study outcomes suggest that AP and CI have good antioxidant activity which represents a low inhibitory concentration 50 in contrast to SM which shows a higher IC 50, thus, we deduced that AP and CI increase the antiradical effect of SM while the mixture AP with CI decreases their effect.

Antioxidant interactions in a mixture can influence its antioxidant activity positively or negatively. Many substances can exhibit an antioxidant activity, these compounds differ inmolecular size, polarity, and solubility and these differences can affect the bioavailability and distribution of each other thus on the antioxidant activity of complex mixtures. in addition, theproblem of antioxidant effect in a mixture is very difficult to solve, particularly that it may depend on: applied method and mechanism of the radical neutralization, composition of the reacting mixture (including: chemical structure, concentration and molecular ratio), applied solvent, treatment of the sample and the reaction time.

All these results obtained in vitro constitute only a first step in the search for biologically active substances of natural origin. From this effect, numerous perspectives can be envisaged:

- The purification and identification of biochemical compounds of each plant
- In vivo studies should be performed to confirm the results obtained so far.
- Carry out other biological tests such as anti-inflammatory, antimicrobial effects, etc.

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