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Regional variation of anti-diabetic and anti-inflammatory activities of *Eucalyptus Sideroxylon*

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I dedicate this work:

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ABSTRACT

Medicinal plants serve as a natural reservoir of bioactive compounds with diverse therapeutic properties. This study aimed to evaluate the biological activities—specifically the antidiabetic and anti-inflammatory effects—of leaf extracts from *Eucalyptus sideroxylon* collected from various regions.

Extraction yields varied significantly, ranging from 2.93% to 39.61% depending on the region. Qualitative phytochemical screening revealed the presence of flavonoids, tannins, saponins, coumarins, free quinones, and cardiac glycosides, while alkaloids, sugars, and proteins were consistently absent across all samples. These findings were supported by quantitative analyses of phenolic compounds, tannins, and flavonoids. The red-flowered of *E. sideroxylon* from the Blida region exhibited the highest total polyphenol content (528.48 mg GAE/g DW) in the methanolic extract, whereas the lowest was recorded in the ethanolic extract from Médéa-Zerouek pink flowers (42.93 mg GAE/g DW). In terms of tannin content, the aqueous extract from Bouguezoul showed the highest level (832.22 mg EAT/g DW), while the ethanolic extract from Blida had the lowest (83.9 mg EAT/g DW). Flavonoid content was highest in the ethanolic extract of *E. sideroxylon* from Médéa-Zerouek (103.42 mg RE/g), and lowest in the aqueous extracts (28.34 mg RE/g).

Anti-diabetic activity, assessed via α-amylase inhibition, revealed that ethanolic extracts demonstrated the strongest inhibitory effects, particularly those from the pink- and white-flowered specimens from Médéa. For anti-inflammatory activity, all extracts—including the reference drug diclofenac—showed dose-dependent increases in erythrocyte membrane stabilization. Among them, the methanolic extract emerged as the most effective and consistent.

Keywords: *Eucalyptus sideroxylon*, extracts, anti-diabetic and anti-inflammatory activity, region.

Résumé

Les plantes médicinales constituent un réservoir naturel de composés bioactifs aux propriétés thérapeutiques variées. Cette étude visait à évaluer les activités biologiques, en particulier les effets antidiabétiques et anti-inflammatoires, des extraits de feuilles d'*Eucalyptus sideroxylon* collectés dans différentes régions.

Les rendements d'extraction ont varié de manière significative, allant de 2,93 % à 39,61 % selon la région. Le criblage phytochimique qualitatif a révélé la présence de flavonoïdes, tanins, saponines, coumarines, quinones libres et hétérosides cardiotoniques, tandis que les alcaloïdes, sucres et protéines étaient systématiquement absents dans tous les échantillons. Ces résultats ont été confirmés par les analyses quantitatives des composés phénoliques, des tanins et des flavonoïdes. L'*E. sideroxylon* à fleurs rouges de la région de Blida a présenté la plus forte teneur en polyphénols totaux (528,48 mg EAG/g MS) dans l'extrait méthanolique, tandis que la plus faible a été enregistrée dans l'extrait éthanolique de Médéa- Zerouek fleurs roses (42,93 mg EAG/g MS). Concernant les tanins, l'extrait aqueux de Bouguezoul a affiché la concentration la plus élevée (832,22 mg EAT/g MS), alors que l'extrait éthanolique de Blida montrait la plus faible (83,9 mg EAT/g MS). La teneur en flavonoïdes la plus élevée a été observée dans l'extrait éthanolique de *E. sideroxylon* de Médéa-Zerouek (103,42 mg RE/g), tandis que les extraits aqueux présentaient les teneurs les plus faibles (28,34 mg RE/g).

L'activité anti-diabétique, évaluée par l'inhibition de l'α-amylase, a montré que les extraits éthanoliques présentaient les effets inhibiteurs les plus marqués, notamment ceux issus des fleurs roses et blanches de la région de Médéa. Quant à l'activité anti-inflammatoire, tous les extraits, y compris le médicament de référence (diclofénac), ont induit une stabilisation des membranes érythrocytaires dépendante de la concentration. Parmi eux, l'extrait méthanolique s'est révélé être le plus efficace et le plus constant.

Mots-clés : Eucalyptus sideroxylon, extraits, activité antidiabétique et anti-inflammatoire, région.

ملخص

تمثل النباتات الطبية خزانًا طبيعيًا للمركبات النشطة بيولوجيًا ذات مجموعة واسعة من الخصائص. هدفت دراستنا إلى تقييم الخصائص البيولوجية، ولا سيما النشاط المضاد لمرض السكري والنشاط المضاد للالتهابات، لمستخلصات أوراق نبات الأوكالبتوس سيدروكسيلون من مناطق مختلفة.

تشير النتائج التي توصلنا إليها إلى أن محصول المستخلص يتراوح بين 2.93% إلى 39.61% في مختلف المناطق، وقد كشف التحليل النوعي لهذه المستخلصات عن وجود مركبات الفلافونويد والعفص والصابونين والكومارين والكينونات الحرة والجليكوسيدات القلبية، وفي المقابل كانت القلويدات والسكر والبروتينات غائبة باستمرار في جميع العينات، وقد تأكد ذلك من خلال التحليل الكمي القائم على تحديد المركبات الفينولية والعفص والفلافونويد. وقد أظهر مستخلص البليدة RF أعلى محتوى من البوليفينول (528.48 ملجم من مكافئ غازي/غرام من المادة الجافة) في مستخلص الميثانولي. في حين لوحظ أدنى مستوى من البوليفينول في مستخلص إيثانولي المدية زرواق ذو الورود الوردية (42.93 ملجم مكافئ غازي/غرام من المادة الجافة) وفيما يتعلق بالعفص كان مستخلص بوغزول المائي هو الأعلى محتوى (832.22 ملجم مكافئ/غرام من المادة الجافة)، بينما كان مستخلص البليدة الإيثانولي هو الأقل (83.9 ملجم مكافئ/غرام من المادة الجافة). كما أن محتوى الفلافونويد كان الأعلى في مستخلص إيثانولي المدية زرواق (103.42 ملجم من الأوكسجين/غرام) والأدنى في المستخلص المائي (28.34 ملجم من الأوكسجين/غرام).

أظهر تقييم النشاط المضاد لمرض السكري ضد تثبيط إنزيم ألفا أميليز أن المستخلص المائي أظهر أعلى استجابة خاصة في المدية ذات الورود الوردية والبيضاء. بالنسبة للنشاط المضاد للالتهابات، أظهرت النتائج التي تم الحصول عليها أن النسب المئوية لتثبيت كريات الدم الحمراء التي تم الحصول عليها في المستخلصات المختلفة والديكلوفيناك تزداد كدالة للتركيز، وكان المستخلص الميثانولي هو المذيب الأكثر فعالية وثباتاً.

الكلمات المفتاحية: أوكالبتوس سيدروكسيلون، مستخلصات، نشاط مضاد للسكري ومضاد للالتهابات، المنطقة

List of abbreviation

Ac = Absorbance of control

AH = Absorbance of test sample heated

As = Absorbance of test sample

AU = Absorbance of test sample unheated

ES: *Eucalyptus sideroxylon*

g : Grammes

µl : Microlitres

min: Minutes

mg : Milligrammes

ml : Millileters

mm : Millimeters

nm : Nanometers

PF: Pink flower

%: Pourcentage.

RBCs: Red blood cells

RF: Red flower

rpm: Revolutions per minute

Y%: Yield

We: Weight of the extracted material (g)

WF: White flower

WHO: World Health Organization

Wp: Weight of the plant biomass (g)

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INTRODUCTION:

The use of medicinal plants or their extracts has become an important subject for research and for pharmaceutical applications **(Affaf et al., 2020)**. Today the field of medicinal and aromatic plants encompasses industries such as perfumery, cosmetics, food processing, and aromatherapy, a subfield of phytotherapy which involves the use of aromas, essences and essential oils as medical treatments **(Kihel & Khelifa, 2023).**

Since ancient times, different parts of plants have been used for medicinal purpose and in recent times, there is increase in the study and search of medicinal herbs by pharmacological researchers. Plants are enriched with different organic compounds, of which many are used for medicinal purposes. These plants contain chemical substances called phytochemicals which are known to produce specific physiological actions in the body. In addition, they form part of a plants natural defense mechanisms agains diseases. They have been implicated in therapy and disease control. Most of them such as phenols and flavonoids have been reported to possess multiple biological properties such as anti-inflammatory, anti-microbial, cytotoxic, anti-tumor and antioxidant activities **(Akpata & Ani, 2022)**.

The attention has been given to the medical characteristics of *Eucalyptus* species, it has been used to treat arthritis, asthma, burns, fever, influenza, sore throat, malaria, wounds and pharyngitis, it has been found that leaves extracts of *Eucalyptus* species have shown many properties and several studies have brought to light the antihyperglycemic, antioxidants, antibacterial activity, ulcer- healing, cytotoxic effect, anti-inflammatory and analgesic effects **(Alghamdi & Ababutain, 2019).**

The *Eucalyptus* tree belongs to the *Myrtaceae* family and it is considered a large aromatic genus that contains about 900 species **(Alghamdi & Ababutain, 2019).** It was introduced to Algeria during colonization between 1854 and 1860. *Eucalyptus* plants are attracting the attention of researchers and ecologists the world over, as they represent a growing source of wood as well as a source of oil used for many purposes. Most *Eucalyptus* are evergreen, but some tropical species lose their leaves at the end of the dry season **(Affaf et al., 2020) .**they among the most widely used plants in phyto-aromatherapy; It has anti-inflammatory properties and has been widely used to treat various inflammatory diseases **(BARAKA, 2019).** Inflammation can be defined as the body's biological defense process against an aggressive agent. anti-inflammatory therapy is designed to control the excessive aspecific reaction of tissues and avoid the transformation of the acute phase of inflammation into a chronic phase.

Corticosteroid agents, which are steroidal anti-inflammatory drugs, all have hormonal activity concerning metabolic regulation. They cause pituitary-adrenal suppression.while non-steroidal anti-inflammatory drugs (NSAIDs) comprise various chemical classes without any steroidal structure **(Professeur, 2005)**

Diabetes mellitus (DM) is a complicated metabolic disorder that has gravely troubled human health and the overall quality of life. It is probably one of the oldest diseases known to man. Although DM is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia, glucose intolerance, insulin resistance, and relative insulin deficiency. Due to their enormous antioxidative and pharmacological significance, medicinal plants are being extensively explored as therapeutic modality of choice against diabetes**(Sabiu & Ashafa, 2016).** *Eucalyptus spp* are classified as herbs with excellent antidiabetic and hypoglycemic properties **(Qabaha et al., 2016)** although various regions around the world have used its hot water decoction as a treatment for diabetes **(Dey & Mitra, 2013).**

The aim of this study is to evaluate the biological, anti-inflammatory and anti-diabetic activities of ethanolic, methanolic and aqueous extracts of two subspecies of *Eucalyptus sideroxylon:* red and white flowered, from different regions (Medea, Blida, Bouguzoul and Khmis Miliana)

CHAPTRE 1:

LITERATURE REVIEW

I.1-The phytotherapy

Etymologically, from the Greek "phyton" meaning plant and "therapein" meaning to heal. Phytotherapy is the use of plants for therapeutic purposes.

I.1.1-Traditional phytotherapy

So-called "traditional" phytotherapy is based on ancestral, empirical uses, based on a holistic approach: it uses the effects of the whole plant on the individual as a whole.

For centuries, medicinal plants have represented the most important therapeutic reservoir. In the absence of scientific tools, a body of knowledge has been built up through observation and experience. Active ingredients were only isolated at the beginning of the 19th century, whereas until then, plants or parts of plants were used as they were, undergoing minor transformations (macerations, infusions, alcoholates...). Similarly, observation of a plant's possible activity on the organism could only be revealed by modification of the patient's symptomatology.

I.1.2-Modern phytotherapy

Based on biochemical knowledge, and seeks to relieve symptoms using active ingredients contained in medicinal plants. It mainly uses products of plant origin obtained by extraction and presented like any other pharmaceutical specialties **(Jorite, 2015).**

I.2-The medicinal plants

In recent decades, medicinal plants have been of great interest as they have been the sources of natural products **(Uyi Ewansiha et al., 2024).**

Plants had been used for the healing of diseases ages ago before the use of recent clinical drugs. Such medicinal plants are also recognized to have therapeutic properties or as precursors for the synthesis of useful drugs. More than 50% of these synthetic drugs are derivative of natural products. These natural products play a crucial role in drug development with the increasing use of chemicals antibiotics ,many pathogens have developed resistance against them, hence there is immense need to develop new anti-agent with improved performance and wide applications **(Kaur et al., 2019).** More than 20,000 plants used worldwide for their medicinal properties, only 2,000 to 3,000 have been scientifically studied according to the World Health Organization (WHO) **(Oullali et Chamek, 2018)**.

I.2.1-Definition

According to the World Health Organization (WHO), medicinal plants are “any plant containing in one or more of its organs substances that may be used for therapeutic purposes or as precursors of substances used for such purposes” **(Barakat et al., 2024).**

I.3-The genus eucalyptus

I.3.1-Origin and definition

Eucalyptus is widely believed to have been discovered during Captain *James Cook's* voyages in the 1770s, during his first voyage plant specimens were collected from Botany Bay under the guidance of Sir *Joseph Banks*. One *eucalypt* specimen was misclassified in 1788 as *Metrosideros* by *Joseph Gaertner*.

On Cook's third voyage, *David Nelson* collected a specimen from Bruny Island (south of Tasmania).

This specimen was later studied by *Charles Louis L’Héritier* de Brutelle, who formally described it in 1788, naming the genus *Eucalyptus* (from Greek eu = well, and calyptos = covered, referring to the flower bud cap) **(John J.W. Coppen (2002).**

Eucalyptus extracts have been approved as food additives and are currently used in various cosmetics formulation, it leaves show many activities such as antioxidant, antiseptic and anti-inflammatory .Besides antimicrobial activity, the essential oil and its constituents also show herbicidal ,insecticidal ,anthelmintics ,anti-tumor and anti-leech activity**(Kaur et al., 2019).**

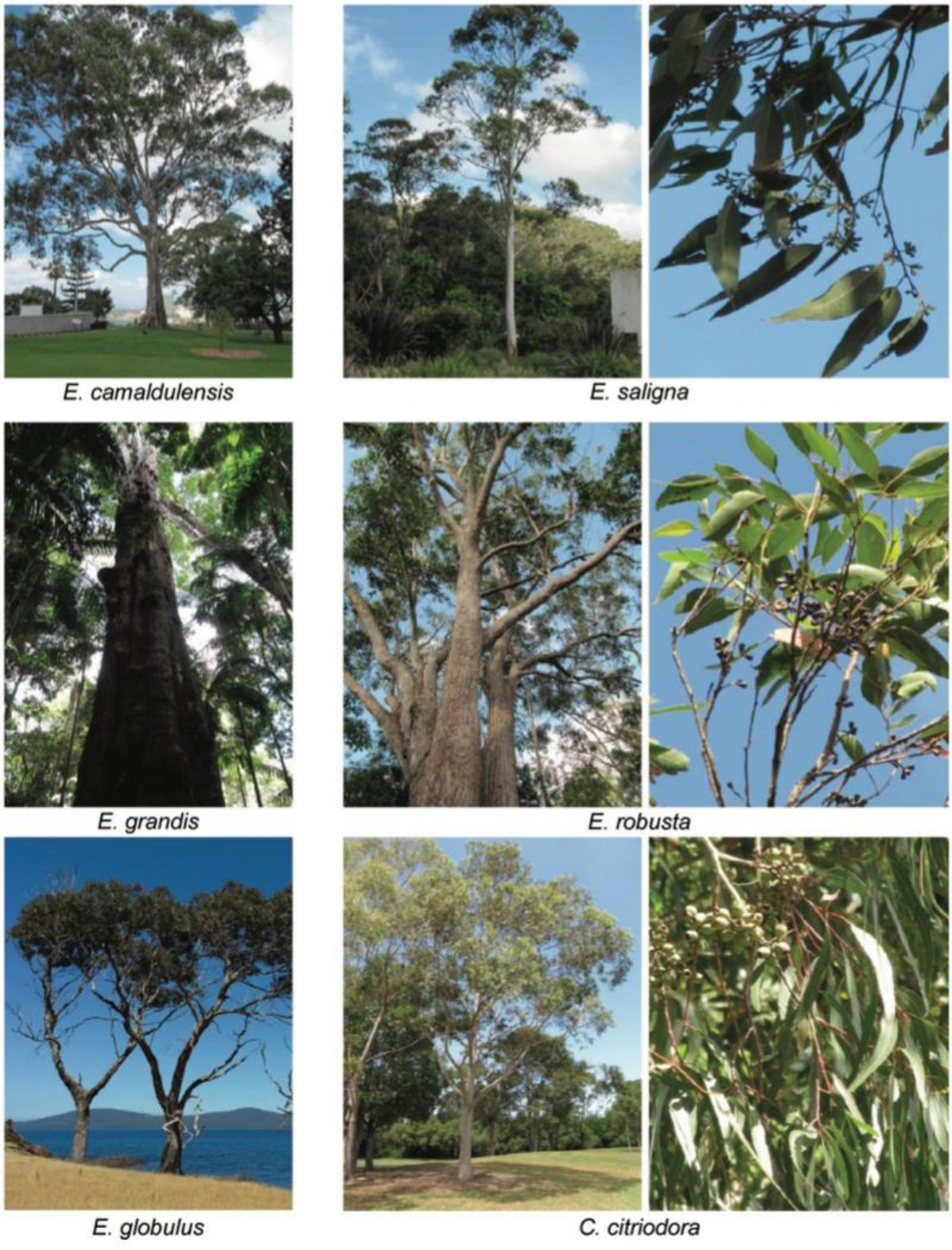


Figure 1: Selected common Eucalyptus species **(Vuong et al., 2015)**

I.3.2-Botanical description

The genus *Eucalyptus*, belongs to *Myrtaceae* and is native to Australia with over 900 species. It has been exported throughout the world. *Eucalyptus* species constitute multiproduct plants par excellence. It is cultivated not just for pulp, timber, and wood, but theirs extracts present significant therapeutic and medicinal characteristics **(Ayed et al., 2024).**

Eucalyptus is a tree 30 to 35 meters tall, with a straight, smooth, greyish trunk, which bears erect branches **(Rameau et al. 1989).** The young leaves are bluish, opposite and narrow tapered on the stem, the adult leaves are dark green, alternating and drooping.

The flowers are visible in spring, born in the axils of the leaves. They are most often in bouquet, sometimes solitary, the calyx with the shape of a bumpy toupie whose wide part is covered by an operculum that detaches at the time of flowering leaving many stamens appear but without petals, nor sepals. The fruit is the angular capsule of the calyx **(Metro, 1970; Beker et al. 1983).**

Eucalyptus trees were introduced to Algeria between 1856 and 1860, and several species have given excellent results in the country's sub-humid and semi-arid regions, mainly below 800 meters altitude and in areas receiving more than 400 mm of annual rainfall **(Kihel & Khelifa, 2023).**

I.3.3-Botanical classification of ES

The classification of Eucalyptus has been determined by **(APG III, 2009)**.

Reigns	*Plantae*
Class	*Magnoliopsida*
Subclass	*Rosidae*
Order	*Myrtales*
Sub-kimgdom	*Tracheobionta*
Division	*Magnoliophyta*
Family	*Myrtacea*
Genus	*Eucalyptus*
Species	*Sidroxylon*

I.3.4-geographical distribution

Eucalyptus plantations cover 13 to 19 million hectares in more than 70 countries **(FAO, 1995).**

The area planted with eucalyptus has increased considerably over the past 20 years, particularly in Asia and South America **(laciau, 2001).**

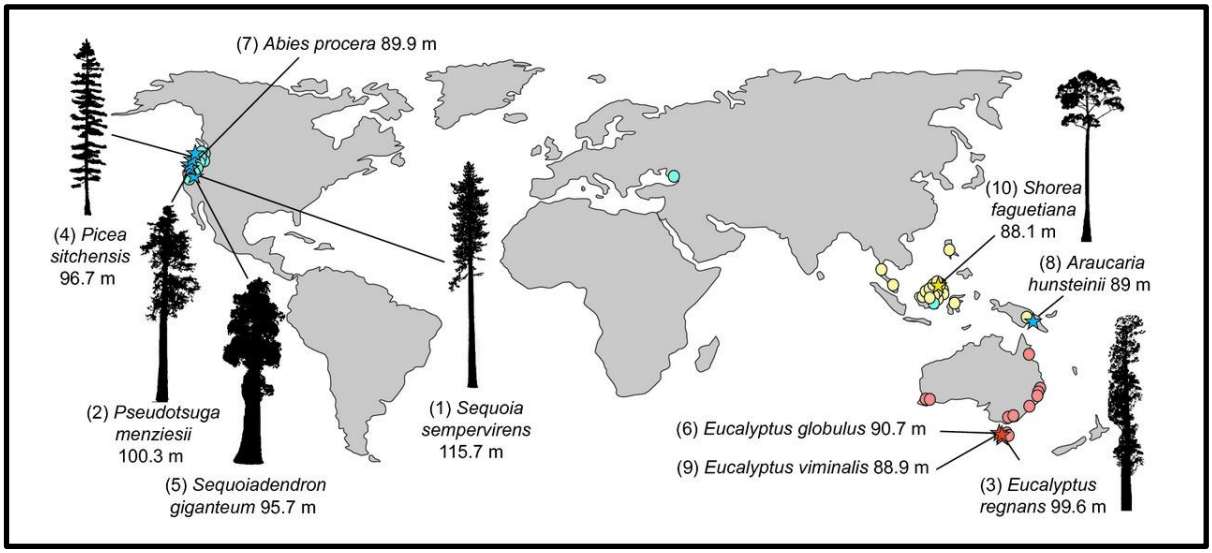


Figure 2: Global distribution of the tree species known to reach 70 m in height **(Tng et al., 2012)**

I 4-Bioactive compounds of medicinal plants

The active ingredient is a molecule present in a medicinal plant or vegetable preparation, responsible for its therapeutic effect, whether it is preventive or curative. Although in small amounts, it is essential for the efficacy of the drug. These principles may be found in different parts of the plant, but not uniformly. Only certain parts may be used depending on the species. These compounds are derived from secondary metabolites, which help the plant to adapt to its environment (light, temperature, insects...)

I 4.1-Flavonoids:

Flavonoids are most of the yellowish pigments (in Latin: flavus = yellow). These compounds are characterized by a benzo-γ-pyrone (or chromone) type structure.

Flavonoids drugs (broadly defined): are derivatives of the phenyl-2 chromane nucleus, they are very common (in higher plants) as heterosides (soluble) (**Guingard, 1996**). They are stored in the vacuolar juice of young organs (leaf skin, fruit film). Obvious role (due to their coloration

in the visible and fluorescence under the effect of solar UV): attraction of pollinating insects. Flavonoids are present in all parts of the upper plants: roots, stems, leaves, flowers, pollen, fruits, seeds, wood. Their main function seems to be the coloration of plants (beyond chlorophyll, carotenoids and betalains) even if their presence is sometimes masked by their presence in forms «leuco», which explains their commercial interests in the food industry**. (àborM et al., 1988).** Flavonoid plants contain a natural yellow polyphenol called FLAVONE. Flavonoids are called which have an unmistakable pharmacological action of bioflavonoids. The most well-known flavonoids (or bioflavonoids) are rutoside and quercetin. They are secondary metabolites of plants, all sharing a basic structure formed by two aromatic cycles linked by three carbons: C6-C3-C6, chain often closed in a hexa- or pentagonal oxygen heterocycles . **(Bruneton, 2009)**.

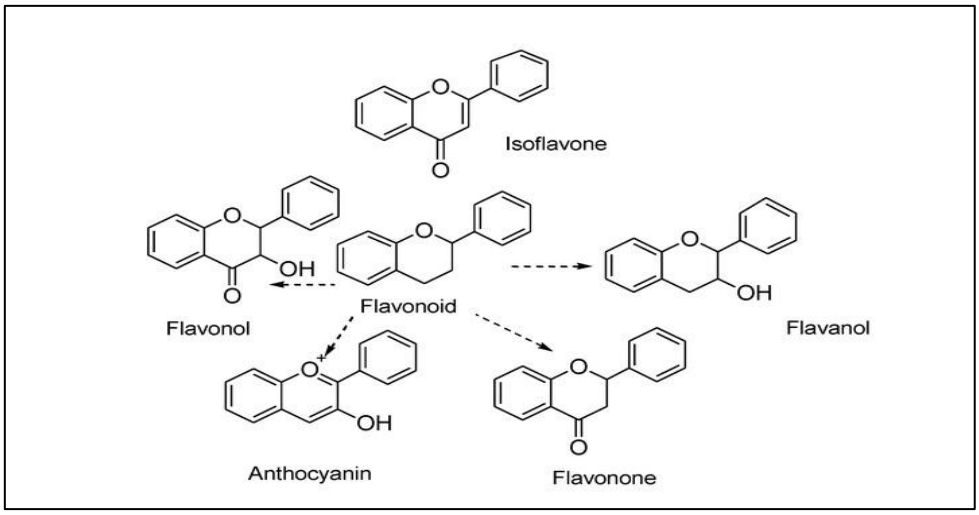


Figure 3: Chemical structure of flavonoids and its different types **(Ullah et al., 2020)**

I 4.2-Tannin

Tannins are secondary metabolites of some vascular terrestrial plants. It is found in all parts of the plant (root, bark, seed shell, cork, fruit: (date, coffee, cocoa&.) Immature, unripe; etc). Tannins are molecules of a phenolic nature (water-soluble polyphenols) **(Conejero, 2013).** But they are particularly abundant in some families such as conifers, Rosacea, Aceraceae, Ericaceae, Fagaceae (quercus) Anacardiaceae, Genariaceae (rhus) **(Ghestem et al., 2001).** These are polyphenolic substances, usually amorphous, and have the effect of turning the skin into leather and precipitating heavy metal salts (lead and mercury, as well as most alkaloids (antidote). There are two groups of tannins: gallic tannins (which are soluble in water) =

(hydrolysable) and cataches (non-hydrolysable)=condensed **(KaramaliKhanbabaee et al., 2001).**

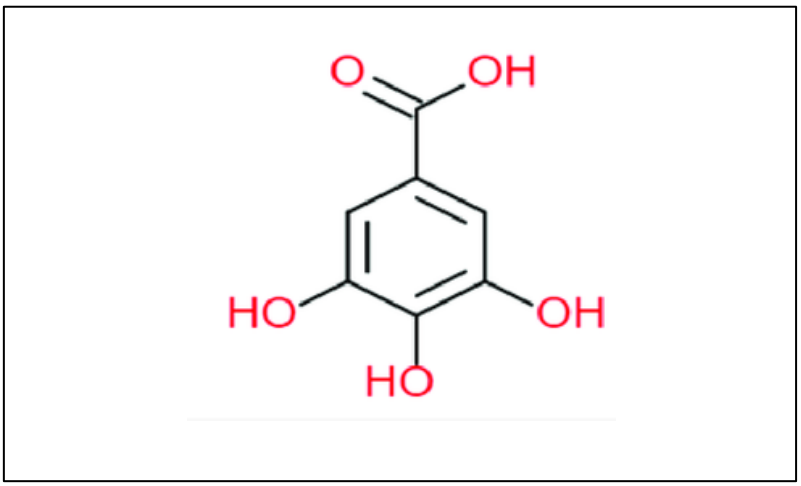


Figure4: Chemical structure of tannin **(Bar et Ganguly, 2022)**

I 4.3-Alkaloids

Alkaloids complex nitrogen compounds of a basic nature, usually having strong physiological effects. Most of them are very active plant poisons with a specific action. Alkaloids are alkaline in nature, found mainly in plants (**volak et stdola, 1983).**

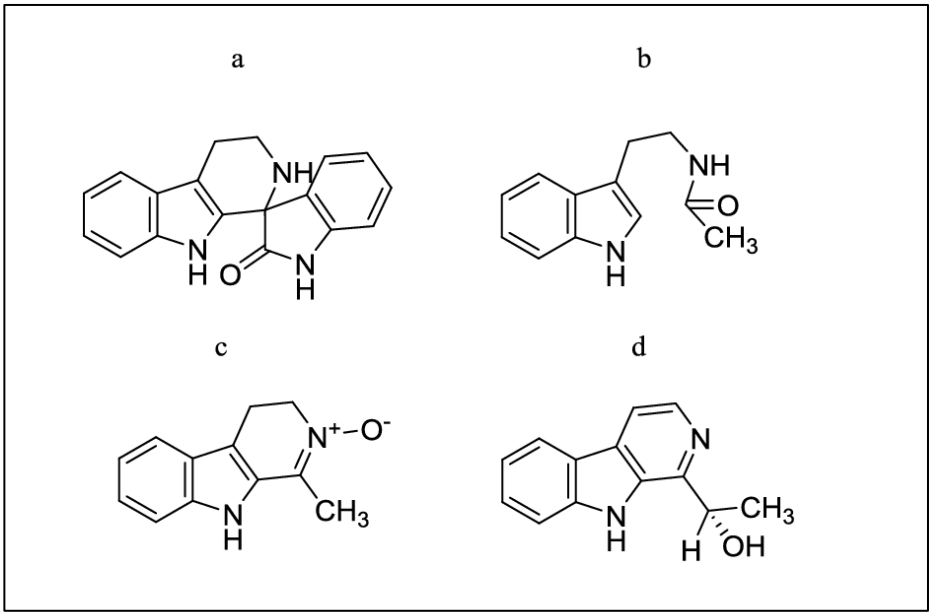


Figure 5 : Structure of four indole alkaloids isolated from D. guianensis. a) Dicorynamine b) N-Acetyltryptamine c) Harmalan-N-oxide d) Cordisinine.**Vanbellingen (2015)**

I 4.4- Polyphenol

Polyphenols are organic compounds characterized by multiple phenol units. They are found in plants, fruits, chocolate, vegetables, and beverages such as tea and wine, and are composed of more than 8,000 different compounds grouped into different classes based on their chemical structure. The two main classes of polyphenol are flavonoids (isoflavones, quercetins, cyanidins, and catechins) and phenolic acids (caffeic and ferulic acids). Research shows that polyphenols may protect against various disorders, including diabetes, osteoporosis, cardiovascular and neurodegenerative diseases, and cancer **(Preedy, 2013).**

I.5-Previous study on biological properties

I.5.1-Yield and phytochemical screening

Alghamdi et Ababutain (2019) Find that the yield of leaves extract of *E. camaldulensis* was higher than the bark extract with values of 40.2 %, 34.8% and 22.2% for ethanol, methanol and water extract, respectively. Than the yield of *Eucalyptus* essential oils varies among the species, with a range of 2 to 4.5%. The *E. dives* has recorded the high yield with 4.5% followed by the *E. citriodora* with 4.3%, and the *E. dives* showed the lowest oil yields with 2%**(Khaoula et al., 2022).** And according to **(Amri et al., 2023)** The hydro distillation of dried leaves yielded 0.7 ± 0.1%, 1.3 ± 0.1%, and 2.9 ± 0.2% of yellow oils for *E. falcata, E. sideroxylon*, and *E. citriodora*, respectively**.**

Uyi Ewansiha et al. (2024) observed the highest yield for aqueous extract 16.08%, and ethyl acetate extract (8.76%) while n- hexane extract had 3.84%. The result did not conform with the reported by **Abdulmumin et al. (2021)**, on though, *Eucalyptus camaldulensis*, aqueous leaves extract 7.30%, ethyl acetate extract 9.29% and n-hexane 1.40%.

The research revealed a diversity of secondary metabolites present in plants such as tannins, alkaloids, and phenols were present in all three solvent extracts; while terpenoids, glycosides and steroids were absent. Saponins and flavonoids were present in water extract, flavonoids were present only in the ethyl acetate extract **(Uyi Ewansiha et al., 2024).** The current study showed the presence of saponins, tannins, flavonoids, carbohydrate and protein compounds in all leaves and bark extracts of *E. camaldulensis* **Alghamdi et Ababutain, (2019).** And **Akpata et Ani (2022)** revealed the presence of phytochemicals in the leaf extract of *Eucalyptus tereticornis* such as saponins, tannins, glycoside, flavonoids, phenols, alkaloids, terpernoids,

and steroids. **Mishra et al. (2010)** showed the presence of flavonoids, terpenoids, saponins and reducing sugars in *Eucalyptus globulus*, also they not having any cardiac glycosides and anthraquinones. Qualitative phytochemical screening of methanol extract of eucalyptus leaves demonstrated the presence of saponins, quinone, flavonoids., carbohydrate, tannin, phenol, fat, flavonoid while protein was absent according to **Kaur et al. (2019).** Also, **Babayi et al. (2004)** demonstrated the presence of saponins, tannins and fat in methanolic extract of eucalyptus leaves except quinone, flavonoids and phenols.

I.6-Anti-diabetic activities

I.6.1-Definition

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia associated with diabetes is linked to long term damage, dysfunction and failure of various organs, particularly the eyes, kidneys, nerves, heart and blood vessels **(Ellitus, 2003).** There are three main types of diabetes: type 1, type 2, gestational diabetes mellitus, and the WHO estimates that diabetes was the seventh leading cause of death in 2019. This is based on a report cited by the International Diabetes Federation **(Momina and Rani, 2020)** .

The study of **Sabiu and Ashafa (2016)** showed that the extract of *E. obliqua* at lower concentrations elicited effects that competed favorably with acarbose (standard drug), in the other hand at higher concentrations where the extract had lower inhibitions and an overall higher IC50 value (2.19 mg mL−1) when compared with the control (0.99 mg mL−1). Also, **Ahlem et al (2009)** showed significant reduction in blood glucose in alloxan- induced diabetic rats with *E. globulus* extract (130 mg/kg body wt) but, since liver glycogen level was not restored, did not recommend the insulin stimulatory effect of eucalyptus. Studies carried out by **Pérez et al (1984)** showed that oral and ip administration of *Eucalyptus* extract (25, 50, 75, 100 g per 250 mL water) in alloxan-induced diabetic mice led to hypoglycemia up to 36% (oral) and 25% (ip), respectively. The study of **Ansari et al. (2021)** showed that *E. citriodora* leaf extract exerts significant antidiabetic effects in vitro and in high-fat-fed rats due to a variety of pancreatic and extra pancreatic actions, they increased glucose uptake by 3T3-L1 cells and inhibited digestion of starch, glucose absorption, DPP-IV enzyme and glycation of protein. And the administration of *E. citriodora* (250 mg/5 ml/kg) for 9 days to HFF obese-diabetic rats improved glycemic control and β-cell function. In STZ-induced diabetic mice, incorporation

of *E. globulus* in diet (62.5 g/kg) and drinking water (2.5 g/L) reduced the hyperglycemia and associated weight loss.**(Dey and Mitra, 2013).**

I.7-Anti-inflammatory activities

I.7.1-Definition

Inflammation is part of a self-protection used by human body against pathogens and helps in healing injured tissues. Pro-inflammatory cytokines as Interlukin-6 (IL-6) and Tumor Necrosis Factor (TNF-α) may cause injury to normal tissues upon triggering of inflammation anywhere in the human body. Excessive production of the pro-inflammatory cytokines may evolve into many chronic inflammatory diseases such as rheumatoid arthritis, asthma, and atherosclerosis. Anti-inflammatory drugs decrease the production of such pro-inflammatory cytokines, and therefore improve the symptoms of inflammation **(Qabaha et al., 2016).**

I.7.2-classification of Inflammation

Stages of inflammation depend on the duration of the process as well as various immune factors and inflammation has been classified into two different classes which are acute and chronic processes .

I.7.2.1-Acute Inflammation

Acute inflammation is a short procedure, lasting from minutes to a few days, and its major features are leakage of plasma proteins or fluid and movement of leukocytes into an extravascular area . These cellular and vascular reactions are intermediated by chemical factors produced from cells or plasma and are responsible for the classic clinical symptoms of inflammation such as swelling, redness, pain, warmth, and loss of function. Even though an inflammatory response can happen in any injurious stimulus, the characteristic of this process is the reaction of vascularized connective tissue . There are three main steps in acute inflammatory responses which include enhanced blood flow to inflame area, followed by vasodilatation and enhanced vascular permeability with leakage of plasma from the microcirculation, and phagocytic leukocyte migration to the surrounding tissue .

I.7.2.2-Chronic Inflammation

Inflammation is a vital response of human immune system. Nevertheless, the state of chronic inflammation can have several secondary consequences in biological response associated with enhanced risk of chronic diseases and disorders. Chronic inflammation in tissue usually happens when inflammatory responses are in the absence of an actual stimulus. It usually occurs through infections that are not resolved either within endogenous protection mechanisms or via some other resistance mechanism from host defences. They can also happen from physicalor chemical agents, which cannot be broken down, as well as from some kind of genetic susceptibility. Persistence of foreign bodies, continuous chemical exposures, recurrent acute inflammation, or specific pathogens are all crucial reasons for chronic inflammation **(Arulselvan et al., 2016).**

I.7.3-Anti- inflammatory drugs

Inflammation has become one of the most serious and interesting areas of research investigation among biomedical researchers. The development of effective, economical non-steroidal anti-inflammatory drugs (NSAIDs) with minimal or no gastrointestinal side effects is important in the pharmaceutical industry's drug discovery process **(Arulselvan et al., 2016).**

I.7.3.1- Steroidal anti-inflammatory drugs (Glucorticosteroid)

Glucocorticoids are secreted by the adrenal glands and have numerous pharmacological properties, many of which are responsible for undesirable effects such as diabetes. They can inhibit all phases of the inflammatory reaction, including both early and late cellular phenomena of inflammation **(Professeur, 2005).**

I.7.3.2-Non-steroidal anti-inflammatory drugs(NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely used therapeutic classes in the world, whether prescribed or self-administered. Their analgesic, anti-inflammatory and antipyretic properties have been recognised since the late 19th century, which explains their widespread use for treating symptoms **(Blain et al., 2000) .**

(Qabaha et al., 2016) evaluate the inhibitory effect of *Eucalyptus spp* extract on Lipopolysacaride (LPS)-induced Interlukin-6 (Il-6) and Tumor Necrosis Factor-α (TNF-α) by polymorphonuclear Cells (PMNCs). They found that the leaves extracts of *Eucalyptus spp* have shown significant reduction in the levels of both Il-6 and TNF-α, due to both anti-inflammatory agent: The Gallic acid which is the major phenolic compound in leaf extracts and Chlorogenic

acid. And according to **(Akpata and Ani, 2022)** the leaf extract of *Eucalyptus tereticornis* and the standard drug diclophenac displayed a concentration dependent activities which were significantly different ($p<0.05$). At the highest concentration of 10 mg/ml, the extract displayed 59.60% and 43.75% inhibition of heat and hypotonicity-induced hemolysis respectively while that of the standard were 76.32% and 56.19% inhibition respectively. The extract displayed 53.87% inhibition of albumin denaturation activity while the standard displayed 71.23% activity. The anti-proteinase activity of the extract was 28.60% while that of the standard was 57.65%. This study has shown that the leaf extract of *Eucalyptus tereticornis* could be used for management and treatment of diseases associated with inflammatory reactions. From the result of **Ashour et al. (2019)** *E. sideroxylon bark* showed potent anti-inflammatory activity through inhibiting heat induced albumin denaturation. The highest inhibition percentage (60.21%) was exhibited at a dose of (500 μg/mL) compared to diclofenac sodium at same concentration which cause 67.29% inhibition and it showed significant membrane stabilization potential superior than the used reference drug at all tested concentration levels.

CHAPTR 2:

MATERIAL AND METHODS

II.1- Presentation of the region

The town of Médéa is located in northern Algeria, about 90 km south of Algiers. Perched at an altitude of around 1,000 meters in the Tellian Atlas Mountains, the region enjoys a Mediterranean climate with a mountainous influence. Winters are cold and sometimes snowy, especially in the higher areas, while summers are hot and dry. Temperatures can drop below 0°C in winter, and rise to 35°C or more in summer.

Boghzeoul is a small town in the wilaya of Médéa, in north-central Algeria. It lies some 25 km south-west of the town of Médéa, close to several villages and the mountains. It's a tranquil region, typical of the Tellian Atlas, with a mountainous Mediterranean climate, cold, wet winters and hot, dry summers. Winter temperatures can sometimes drop below 0°C, especially in the higher areas, and snow is a possibility. In summer, temperatures can reach 30-35°C. Boghzeoul is a predominantly agricultural region.

Khemis Miliana is in northern Algeria, in the wilaya of Aïn Defla, about 75 km west of Algiers. It lies on the Mitidja plain, at the foot of the Tellian Atlas Mountains. Winters are mild and rainy, while summers are hot and dry. Winter temperatures generally fluctuate between 8 and 12°C, while summer temperatures can exceed 30°C. Khemis Miliana is an agricultural region with fertile soils.

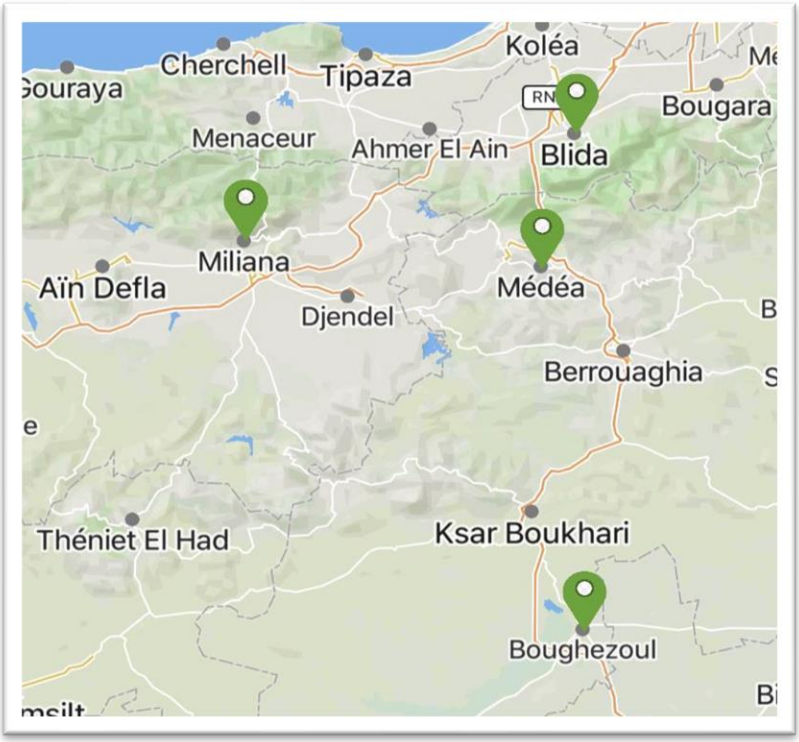


Figure 6: Mapcarta 2025

The wilaya of Blida is located in northern Algeria, at the foot of the Tellian Atlas, about 45 km southwest of Algiers. Nicknamed “the city of roses”, the Blida region enjoys a moderate Mediterranean climate. Summers are hot and dry, while winters are mild and wet. Temperatures average between 8°C in winter and 35°C in summer. The town is protected from the north wind by the Djurjura mountains, and enjoys a lush, green landscape.

II.2- Material used

II.2.1- Plant material

We use two subspecies of Eucalyptus sideroxylon: one with Red flowered and the others with White flowered. Leaves that belong to both subspecies were harvested in November and December 2024 from different regions such as: Bouguezoul, Medea, Khemis Miliana and Blida. The identification of the subspecies was carried out by Dr. Kirouani A (Researcher at University of Medea).

Collected leaves were cleaned and air-dried during 15 days and stored at room temperature and protected from the direct light. Leaves should be reduced into fine powder at laboratory of agronomy using grinder and then filtered to obtain a fine texture with a reduced surface area.

II.2.3-Methods

II.3.1- Preparation of extracts: methanolic, ethanolic and aqueous extract

Operatory mode

This was achieved following the method of **(Alghamdi and Ababutain, 2019).** First ,40g of crushed plant material should be soaked in 200 mL of solvents (methanolic, ethanolic and aqueous) for two days with shaking to ensure proper extraction of the active ingredients from the sample .The mixtures will be then filtered on Whatman No.1 filter paper. The methanol, ethanol and aqueous filtrates will be concentrated to dryness using rotary evaporator to obtain the crude methanol and ethanolic extracts. The aqueous extract should be dried and grinded to obtain a dry powder extract. The respective extracts obtained was transferred to sterile petri dishes and kept in a refrigerator 4°C until use.

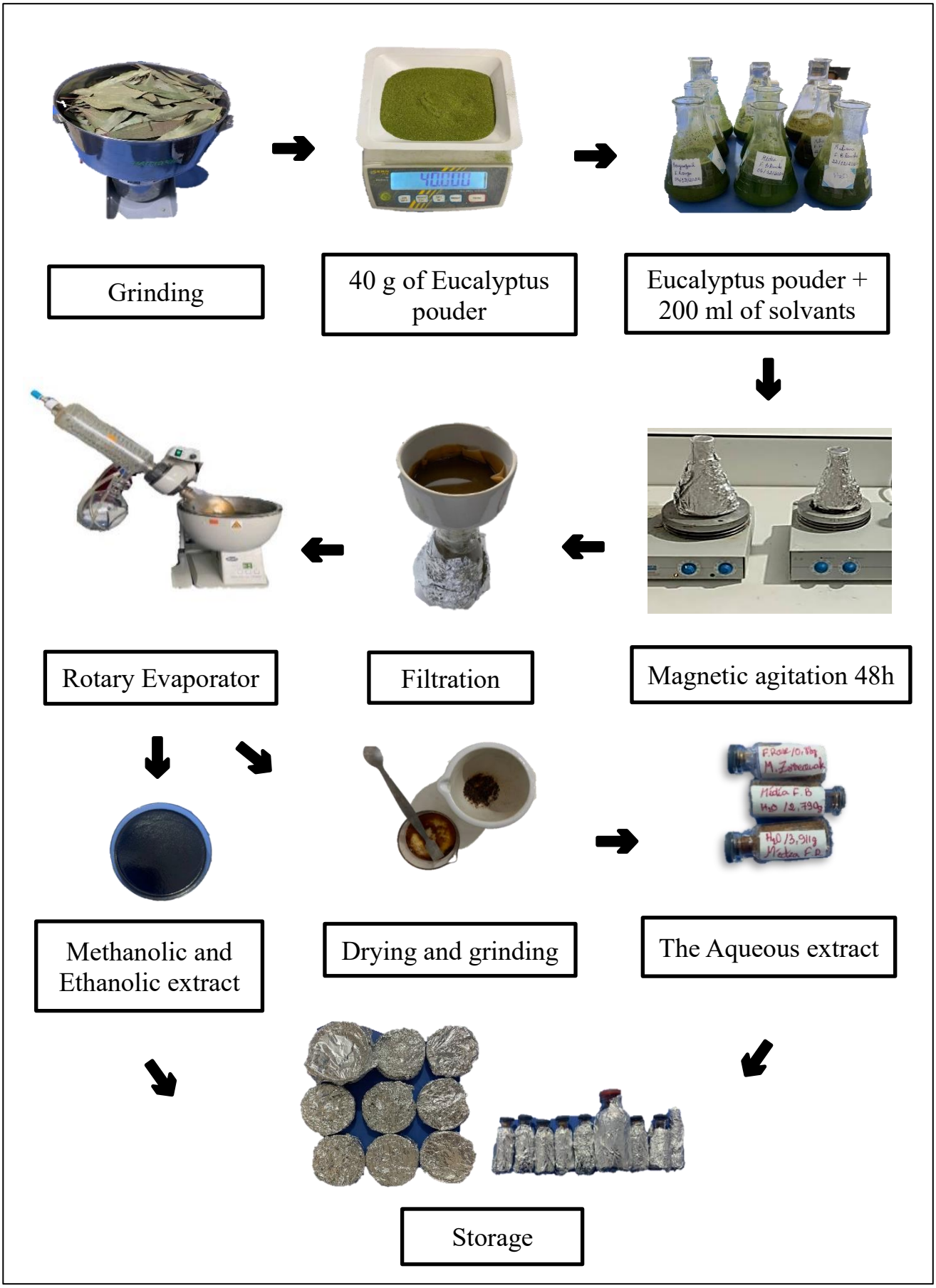


Figure 7: Preparation of methanolic, ethanolic and aqueous extract

II.3.2-Calculation of yield

It’s the ratio between the weight of the extracted material and the weight of the plant biomass to be processed, expressed as a percentage of plant matter. It varies according to factors such as plant age, harvesting period and drying.

Y% = We / Wp * 100

Y: yield (%)

We: the weight of the extracted material (g)

Wp: the weight of the plant biomass (g)

II.3.3-Phytochemical Analyses

II.3.3.1-Phytochemical Screening

Phytochemical screening identifies the different families of chemical compounds present in a sample. The characterization methods primarily involve qualitative analysis, which may rely on either the formation of insoluble complexes through precipitation reactions or the formation of colored complexes through staining reactions.

A. Detection of Alkaloids

Alkaloids were detected using Dragendorff's reagent. A 0.1 g residue was dissolved in 6 mL of 60% ethanol. Adding two drops of Dragendorff's reagent to the alcoholic solution produced a red-orange or reddish-brown precipitate, indicating a positive test.

B. Detection of Saponins

Three milliliters (3 mL) of the extract's aqueous solution were mixed with 10 mL of distilled water in a test tube. The test tube was stoppered and vigorously shaken for 5 minutes, left to stand for 30 minutes, and observed for honeycomb froth, indicating saponins. The presence of saponins is confirmed if the index exceeds 100.

C. Detection of Tannins

One milliliter (1 mL) of each extract was mixed with 1 mL of water and 1–2 drops of 1% FeCl3 solution. A dark green or blue-green coloration indicated tannins. Dark green suggested catechin tannins, while blue-green indicated gallic tannins.

D. Detection of Flavonoids

Two milliliters (2 mL) of the extract were mixed with a few drops of concentrated HCl and about 0.5 g of magnesium metal. A rose-orange coloration indicated a positive reaction.

E. Detection of Reducing Compounds (Sugars)

Two milliliters (2 mL) of the extract were placed in a test tube, and 2 mL of Fehling's solution were added. The mixture was boiled in a water bath for 8 minutes. A brick-red precipitate indicated the presence of reducing compounds.

F. Detection of Proteins

Proteins were identified using the biuret test. A residue aliquot dissolved in 2 mL of 20% aqueous NaOH was mixed with 2–3 drops of 2% aqueous CuSO4. A violet coloration indicated a positive reaction.

G. Detection of Coumarins

Coumarins were identified via a lactonic cycle reaction. Two milliliters (2 mL) of ethanolic solution from each residue were placed in test tubes. To one, 0.5 mL of 10% NaOH was added. The tubes were heated in a water bath to boiling, then cooled. Four milliliters (4 mL) of distilled water were added to each. A transparent solution compared to the control indicated a positive reaction. Acidifying the clear solution with concentrated HCl caused it to lose its yellow color and precipitate.

H. Detection of Free Quinones

Two milliliters (2 mL) of extract were mixed with a few drops of 1% NaOH. A red or violet coloration indicated a positive result.

I. Detection of Cardiac Glycosides

Two milliliters (2 mL) of chloroform were added to 1 mL of the extract. A reddish-brown coloration after adding H2SO4 indicated cardiac glycosides.

II.3.3.2-Quantification of Bioactive Molecules

II.3.3.2.1-Total Polyphenols

a) **Principle**

The total concentration of phenolic compounds in the methanolic, ethanolic and aqueous extracts of two *Eucalyptus* subspecies was measured spectrophotometrically

using the Folin-Ciocalteu method **(Skerget et al., 2005)**. This reagent, which is yellow and composed of phosphotungstic acid and phosphomolybdic acid, is reduced during the oxidation of phenolic compounds, forming blue oxides of tungsten and molybdenum. The intensity of the color, with maximum absorbance between 725 nm and 760 nm, is proportional to the amount of oxidized phenolic compounds in the extracts **(Boizot and Charpentier, 2006).**

b) Procedure

100 µL of the extract (1 mg/mL) was mixed with 500 µL of Folin-Ciocalteu reagent (pre-diluted 10 times). The solutions were mixed for 1 minute then we added 2 mL of 2% sodium carbonate solution (Na_2CO_3). After agitation, the mixture was incubated for 30 minutes in the dark. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as a standard to create a calibration curve. Total phenol content was expressed as milligram per gram of dry weight (mg GAE/g DW).

II.3.3.2.2-Condensed Tannins

a) **Principle**

Condensed tannin content was determined using the method described by **Julkunen-Titto (1985).**

b) Procedure

50 ml of each extract was added to 1500 µL of 4% vanillin solution in methanol. The resulting mixture was vigorously shaken and 750 µL of concentrated hydrochloric acid added. The resulting mixture was left to react at room temperature for 20 min. Absorbance was measured at a wavelength of 550 nm against a blank solution of 4% vanillin in methanol. A stock solution of tannic acid was used as a reference standard for establishing the calibration curve and for quantifying condensed tannin content expressed in milligram equivalents of tannic acid per gram of dry matter (mg EAT/g dry matter) **(Julkunen-Titto 1985).**

II.3.3.2.3-Flavonoid

a) Principle:

The flavonoid content in the extracts was determined spectrophotometrically using the method described by **Quettier-Deleu et al. (2000),** which is based on the formation of a flavonoid-aluminum complex with a maximum absorbance at 430 nm. Quercetin was used to prepare the calibration curve.

b) Procedure:

1 mL of the diluted sample was mixed with 1 mL of a 2% methanolic aluminum chloride solution.After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Milton Roy 601 UV–Vis spectrophotometer and the flavonoids content was expressed in milligrams per gram of rutin equivalent (mg RE/g).

II.4-The study of biologicals activities in vitro

II.4.1-Anti-diabetic activities

II.4.1.1.Alpha Amylase inhibition

Operatory mode

The α-amylase inhibitory activity and the mode of inhibition were evaluated, following the method of **Sabiu et al. (2016)** with slight modification, we prepared varying concentrations of the extract (5-7.5-10-12.5 mg in 1,5ml of tampon) and 500 µl of each was mixed with 500 µl of 0.02 M sodium phosphate buffer (PH 6,09) containing 500 µl of alpha amylase solution and 500 µl of amidon and incubated in test tubes at 37 °C for 10 min .then 1000 µl of DNS was added to each tube. The mixtures were then incubated in a boiling water bath for 5 min .the reaction mixtures were then diluted with distilled water (10 ml) and we read the absorbance measured at 504nm using a spectrophotometer and the values compared with blanc, which containe 500 µl of the buffer instead of the of the enzyme. A control reaction contained same solution except the extract. The experiments were conducted in triplicate, and the α-amylase inhibitory activity was expressed as %inhibition. The concentration of the extract causing 50% inhibition (IC50) of α-amylase activity was estimated from its standard calibration curve.

% Inhibition = [(Ac -As)/Ac] × 100

For the kinetic experiments, the extract was taken at its IC50 value and incubated with α-amylase, while the starch concentration (substrate) was varied from 0.3 to 5 mg mL^{-1}, as previously described. The amount of reducing sugars released was determined spectrophotometrically using a glucose standard curve and converted into reaction velocities (v). The Lineweaver–Burk double reciprocal plot (1/v versus 1/[S]) was constructed. Importantly, the Km and Vmax values were determined only for the region exhibiting the highest inhibitory effect at low extract concentration, corresponding to the most effective inhibition.

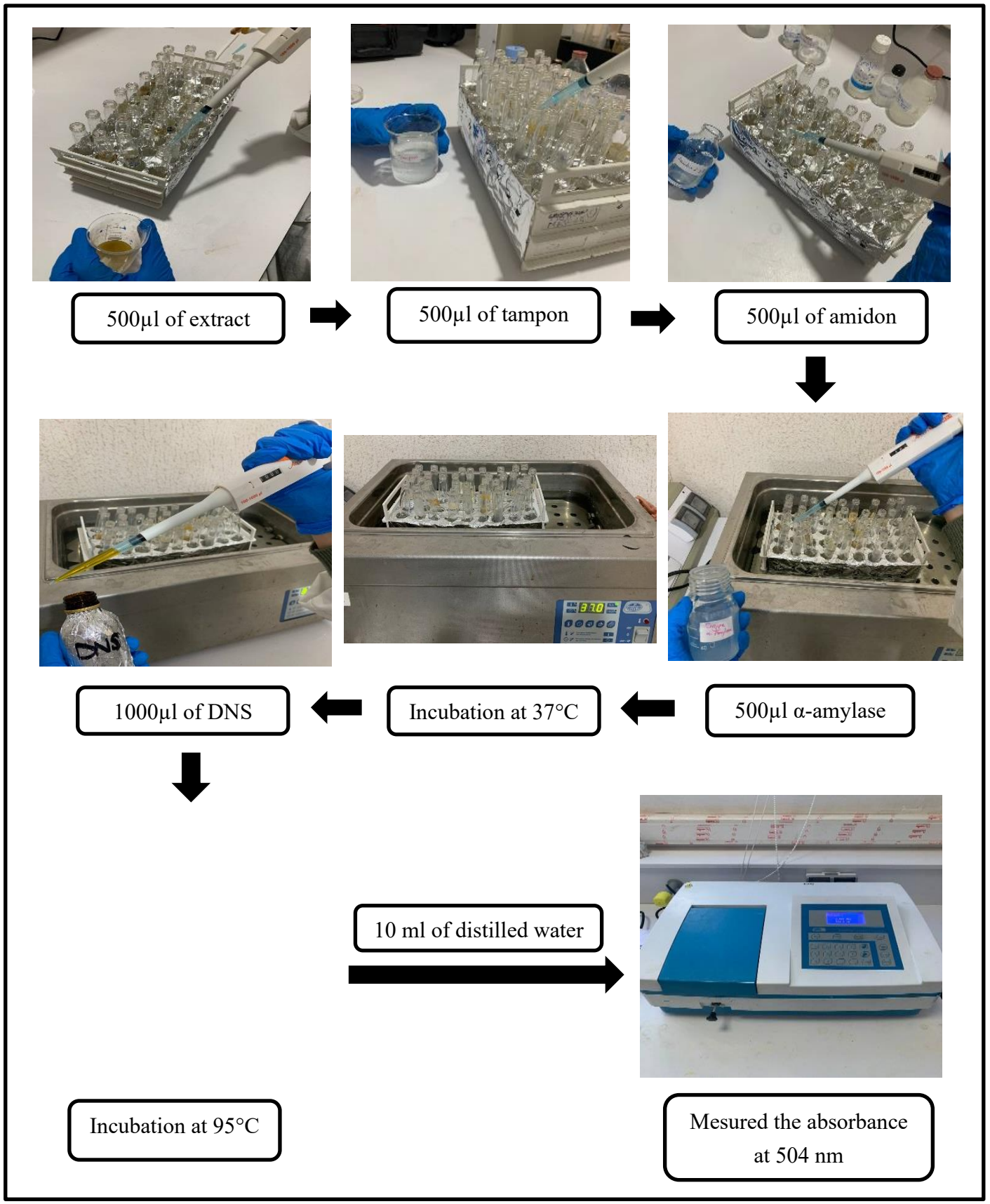


Figure 8: Alpha Amylase inhibitory potentials of methanolic, ethanolic and aqueous extract of *Eucalyptus sideroxylon*

II.4.2-Anti-inflammatory activities

II.4.2.1-Membrane stabilizing activity

The stability of the erythrocyte membrane was assessed by subjecting the red blood cells (RBCs) to two different types of cell lysis: hypotonic and heat induced.

II.4.2.1.1-Preparation of blood cell suspension

We use the method of **Oyedapo et al. (2010)** with same modification. Fresh human blood samples were collected in ethylene diamine tetraacetic acid (EDTA) bottles and centrifuged at 3,000 rpm for 10 minutes. The resulting supernatant was carefully aspirated using a Pasteur pipette. The packed red blood cells were then washed with an isotonic buffered solution (154 mM NaCl in a 10 mM sodium phosphate buffer solution at pH 7.4) and centrifuged at 3000 rpm for 10 minutes, repeating this process until the supernatant was clear. The resulting pellet was used to prepare a 2% (v/v) stock suspension of erythrocytes (RBCs), which was subsequently used.

II.4.2.1.2-Hypotonic solution-induced hemolysis

The analysis was performed according to the protocol of **Oyedapo et al. (2010)** and **Mohammad et al. (2014).** 0.5 ml of stock erythrocyte (RBC) suspension was mixed with 4.5 ml of hypotonic solution (5 mM NaCl in 10 mM sodium phosphate-buffered saline solution, pH 7.4), containing either 1.0 ml of the extract (0.1–0.5–1 mg/ml) or diclofenac (standard drug). For the control sample, 0.5 ml of RBCs was mixed with hypotonic buffered saline alone. The resulting mixture was then incubated at 37 °C for 10 minutes and subsequently centrifuged at 3000 rpm for 10 minutes prior to an absorbance reading at 540 nm using a spectrophotometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

%inhibition of hemolysis = (Ac-As/Ac)*100

II.4.2.2-Heat-induced hemolysis

In this assay, 4 ml of an isotonic buffer was mixed with 0.5 ml of the extract/declofenac solution. This mixture was then placed in two duplicate sets of centrifuge tubes. Next, 0.5 ml of a 2% erythrocyte suspension was added to each tube and mixed gently by inversion. For the control, 0.5 ml of 2% RBCs was mixed with 4.5 ml of hypotonic solution. One pair of tubes

was incubated at 54 °C for 20 minutes in a water bath and the other pair was kept at 0–5 °C in an ice bath. The reaction mixture was then centrifuged for 5 minutes at 3000 rpm and the absorbance of the released hemoglobin in the supernatant was measured at 540 nm using a spectrophotometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

%inhibition of hemolysis = (Ah-Au /Ac-Au)*100

Data Analysis

The variable data were subjected to a two-way analysis of variance (ANOVA) using the 'Factorial ANOVA' option included in STATISTICA software, Version 8.0. For the variables (Total polyphenol content, Total flavonoid content, Condensed tannin content, Antidiabetic activity via Alpha-Amylase inhibition method, Anti-inflammatory activity via Membrane Stabilizing Activity and Heat-Induced Hemolysis methods), the effects analyzed were species, extract, and the interaction "Extract × Species" but only the interaction will be discussed.

Mean comparisons for all studied variables were conducted based on the Least Significant Difference (LSD) at the 5% level, using Fisher's test. Probability values were interpreted as follows:

 0.01<P≤0.050.01 < P \leq 0.05: significant

 0.001<P≤0.010.001 < P \leq 0.01: highly significant

 P≤0.001P \leq 0.001: very highly significant

Graphs were generated using Excel 2013 and STATISTICA Version 8.0.

CHAPITR 3:

Results and discussion

III -1. Phytochemical study of methanolic, ethanolic and aqueous extracts

III -1.1. Yield

In this study, we determined the extraction yields of our crude *E. Sideroxylon* leaf extracts from different regions, which were prepared using the maceration method. The results are shown in the tables below.

Table 1: Regional variation of the yield of different Eucalyptus sideroxylon sub-species

Extract	Sub-species	Blida	Miliana	Bouguezoul	Medea
Methanolic extract	Red-flowered Es	8.886 %	22.277 %	21.057 %	9.232 %
	White-flowered Es	20.494 %	24.555 %	25.208 %	23.745%
	Pink-flowered Es	-	-	-	14.173 %
Ethanolic extract	Red-flowered Es	28.27 %	23.952 %	39.612 %	21.452 %
	White-flowered Es	6.462%	26.952 %	33.120 %	28.952 %
	Pink-flowered Es	-	-	-	31.27 %
Aqueous extract	Red-flowered Es	4 %	5.55 %	12.74 %	9.77 %
	White-flowered Es	3.12 %	6.64 %	6.64 %	6.97 %
	Pink-flowered Es	-	-	-	2.93 %

The data highlights notable variations in extract yields of different *Eucalyptus sideroxylon* sub-species across four regions—Blida, Miliana, Boughezoul, and Medea—using methanolic, ethanolic, and aqueous solvents. Among the three solvents, ethanolic extracts consistently exhibit the highest yields, especially for the red-flowered sub-species collected from Boughezoul, where it reaches 39.612%. This is followed by the same sub-species from Miliana and Blida with 23.952% and 28.27%, respectively. The white-flowered sub-species also performs well in ethanolic extraction, particularly in Medea (28.952%) and Boughezoul (33.120%).

Methanolic extraction presents intermediate yields across sub-species and regions. White-flowered samples produce relatively high yields in Boughezoul (25.208%) and Miliana (24.555%), suggesting a good compatibility with this solvent. Red-flowered samples show lower methanolic yields by comparison, except in Miliana (22.277%). The pink-flowered sub-species is only represented in Medea, where it delivers a moderate methanolic yield of 14.173%, indicating solvent selectivity.

Aqueous extraction yields are universally the lowest, regardless of sub-species or location. The best performance occurs with red-flowered samples from Boughezoul at 12.74%, while all other values remain below 10%. This suggests that water is the least effective solvent for extracting bioactive compounds from these sub-species under the tested conditions.

Sub-species behavior also varies. Red-flowered Eucalyptus responds best to ethanol, particularly in Boughezoul, while white-flowered variants yield consistently high values in both methanolic and ethanolic extracts. The limited data on pink-flowered samples, confined to Medea, still reveal strong ethanol performance at 31.27%, implying its potential if further studied.

Geographically, Boughezoul stands out as the most productive site, consistently delivering the highest yields across different sub-species and solvents. Miliana and Medea follow, with Blida generally yielding the lowest, especially in aqueous extraction. This emphasizes the influence of regional factors—possibly climate, soil, or altitude—on extraction efficiency.

The current results align closely with those reported by Khedachi and Merrah (2024), reinforcing consistency in extraction yield patterns among *Eucalyptus* species. Compared to the findings of Shayoub et al. (2015), who reported yields of 9.34% for ethanolic and 6.54% for aqueous extracts from *Eucalyptus camaldulensis*, the present ethanolic yields—particularly those exceeding 20% and even reaching 39.612%—are substantially higher. This suggests that *Eucalyptus sideroxylon*, especially the red-flowered sub-species from Boughezoul, may possess a richer phytochemical profile under ethanol than *E. camaldulensis*.

When compared to aqueous extraction yields from other studies, our findings also show a similar pattern of limited efficiency. For instance, Uyi Ewansiha et al. (2024) reported a notably high aqueous yield of 16.08%, while Abdulmumin et al. (2021) found 7.30% for the same species. These values are relatively close to our maximum aqueous yield of 12.74% from red-flowered samples in Boughezoul, but most of our values remained below this threshold. These variations further confirm that aqueous solvents generally yield less, although certain environmental or biological factors might occasionally enhance extraction.

Such differences across studies can be attributed to several critical parameters, including regional climate factors like temperature and humidity, as well as biological variables such as plant age, the timing of harvest, and drying methods. These conditions significantly influence the concentration and solubility of bioactive compounds, thereby affecting overall extract yield. The consistency of high ethanol yields in our study, compared with more moderate aqueous

outcomes in other works, underscores both the importance of solvent selection and the strong extractive potential of *E. sideroxylon* under favorable environmental conditions.

III -1 .2. Phytochemical screening

The phytochemical screening across ethanolic, methanolic, and aqueous extracts of red-flowered and white-flowered *Eucalyptus sideroxylon* reveals both shared and distinct profiles in secondary metabolite distribution among regions.

Alkaloids are notably absent in all samples from Blida, Medea, Boughezoul, and Miliana across the three solvents. Only in Medea Zerouek is their presence indicated, but exclusively in the white-flowered variant, suggesting a limited and location-specific occurrence. This contrasts with the ubiquitous presence of saponins and tannins, which appear consistently in both subspecies and in all regions and solvents, underlining their structural stability and prevalence in *E. sideroxylon* regardless of environmental or extraction conditions.

Flavonoids display a more selective presence. They are entirely absent in Medea across all extracts, highlighting a possible environmental limitation in biosynthesis. In contrast, both subspecies from Blida are rich in flavonoids in every solvent. Boughezoul and Miliana show partial detection, varying by subspecies and solvent, suggesting that regional factors and flower type influence flavonoid accumulation.

Sugars and proteins are undetected in all standard regions, regardless of extraction method or subspecies, implying either minimal concentrations or inefficiency of the employed methods for extracting these primary metabolites. This absence contrasts sharply with the consistent detection of more structurally complex phytochemicals such as coumarins and free quinones, which are present in all regions and extracts, indicating robust biosynthetic expression and extractability.

Cardiac glycosides show a solvent-dependent pattern. They are absent in methanolic extracts but present in both ethanolic and aqueous forms, uniformly across all regions and subspecies. This solvent specificity suggests greater solubility or stability of these compounds in polar protic solvents like ethanol and water. Notably, Medea Zerouek's red-flowered variant contains cardiac glycosides in all solvents, reinforcing the unique chemical richness of this site.

Table 2: Results of the chemical screening of the Ethanolic,Methanolic and Aqouas extracts for the regions of Blida,Medea,Bougazoul,Miliana red flower and white flower.

Extract	Methanolic		Ethanolic		Aqueous	
Sub-species	WF-Es	RF-Es	WF-Es	RF-Es	WF-Es	RF-Es

Alkaloids						
Blida	-	-	-	-	-	-
Medea	-	-	-	-	-	-
Bouguzoul	-	-	-	-	-	-
Miliana	-	-	-	-	-	-
Medea Zerouek	/	-	/	-	/	-
Tannin						
Blida	+	+	+	+	+	+
Medea	+	+	+	+	+	+
Bouguzoul	+	+	+	+	+	+
Miliana	+	+	+	+	+	+
Medea Zerouek	/	+	/	+	/	+
Flavonoid						
Blida	+	+	+	+	+	+
Medea	-	-	-	-	-	-
Bouguzoul	-	+	+	-	+	+
Miliana	+	-	-	-	-	-
Medea Zerouek	/	-	/	-	/	-
Flavonoid						
Blida	+	+	+	+	+	+
Medea	-	-	-	-	-	-
Bouguzoul	-	+	+	-	+	+
Miliana	+	-	-	-	-	-
Medea Zerouek	/	-	/	-	/	-
Sugar						
Blida	-	-	-	-	-	-
Medea	-	-	-	-	-	-
Bouguzoul	-	-	-	-	-	-
Miliana	-	-	-	-	-	-
Medea Zerouek	/	-	/	-	/	-
Proteins						
Blida	-	-	-	-	-	-
Medea	-	-	-	-	-	-
Bouguzoul	-	-	-	-	-	-
Miliana	-	-	-	-	-	-
Medea Zerouek	/	-	/	-	/	-
Coumarins						
Blida	+	+	+	+	+	+
Medea	+	+	+	+	+	+
Bouguzoul	+	+	+	+	+	+
Miliana	+	+	+	+	+	+
Medea Zerouek	/	+	/	+	/	+
Free Quinones						
Blida	+	+	+	+	+	+
Medea	+	+	+	+	+	+
Bouguzoul	+	+	+	+	+	+
Miliana	+	+	+	+	+	+
Medea Zerouek	/	+	/	+	/	+
Cardiac Glycisides						
Blida	-	-	+	+	+	+
Medea	-	-	+	+	+	+
Bouguzoul	-	-	+	+	+	+
Miliana	-	-	+	+	+	+
Medea Zerouek	/	+	/	+	/	+

Overall, ethanolic and aqueous extracts tend to yield a broader spectrum of bioactive compounds, particularly cardiac glycosides, while methanol extracts fewer of them, especially in the case of flavonoids and glycosides. The red-flowered subspecies generally demonstrates

richer phytochemical content than the white-flowered form, particularly in Boughezoul and Medea Zerouek. Among the regions, Blida and Boughezoul consistently reveal the presence of most secondary metabolites across solvents, reflecting stable chemical profiles favorable for therapeutic potential. These differences further underscore the influence of regional microclimates and plant physiology on secondary metabolite composition.

Compared to our results, where certain phytochemicals such as alkaloids, sugars, and proteins were generally absent or inconsistently detected across regions and solvents, compounds like tannins, glycosides, and flavonoids showed a notably strong and consistent presence. This aligns with the findings of Akpata & Ani (2022), who also reported tannins and glycosides in strong concentrations (+++), although their study observed moderate levels of alkaloids (++), which were largely absent in our screening—detected only in limited form in the white-flowered subspecies from Medea Zerouek.

Kaur et al. (2019) observed that while both their tested samples shared the presence of tannins and the absence of proteins, there was a variation in flavonoids and quinones depending on the plant part and extraction technique. This variation is echoed in our findings, where flavonoids are present in some regions such as Blida and Boughezoul but absent in others like Medea, and their detection appears to depend on both subspecies and solvent used. Similarly, free quinones were consistently present in our samples, suggesting strong extraction efficiency or widespread distribution in *E. sideroxylon* tissues.

In contrast, Kwansa-Bentum et al. (2023) found that methanol extracts contained alkaloids but lacked flavonoids and tannins, indicating a narrow phytochemical spectrum. Our methanolic extracts, however, were richer in tannins and, in some cases, flavonoids, particularly in Blida and Miliana. This difference may be attributed to species-specific chemical compositions or the influence of regional factors on metabolite accumulation.

Mishra et al. (2010) reported the presence of flavonoids and reducing sugars in *Eucalyptus*, which partly corresponds with our results. While we observed flavonoids in several extracts—especially ethanolic ones—reducing sugars were not detected in any sample, regardless of region or solvent. This discrepancy may reflect differences in analytical sensitivity, plant species, or stages of leaf maturity at collection.

Altogether, while our findings are in partial agreement with existing literature regarding the consistent presence of tannins, glycosides, and flavonoids, variations in alkaloid, sugar, and

protein detection highlight the significant influence of methodological, botanical, and environmental factors on phytochemical expression and extractability.

III.1.3-Quantification of bioactive molecules

The polyphenol content in our three extracts (ethanol, methanol and aqueous) was determined from a calibration curve developed with different concentrations (0.3, 0.75, 1 mg/ml) of a standard gallic acid solution. Our calibration curve includes a linear regression equation ($y = 2.8572x$) and a correlation coefficient of ($R^2 = 0.9727$), which are shown in Figure 9.

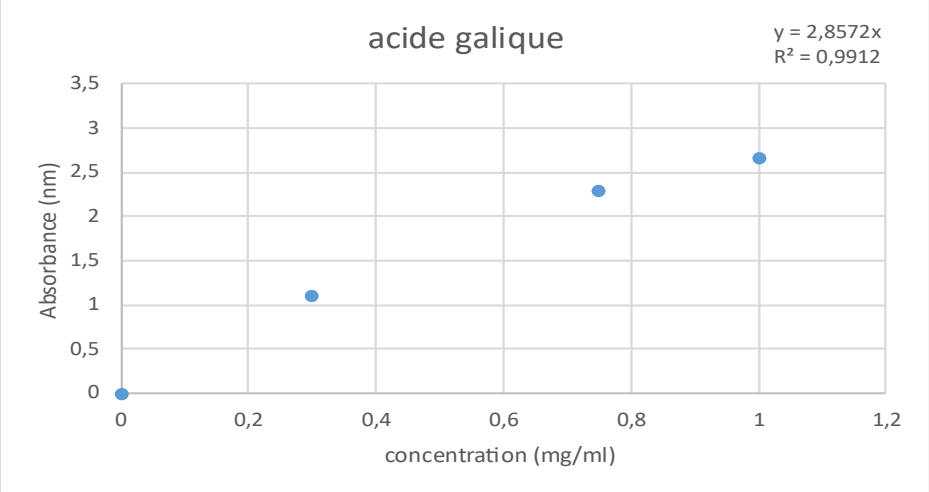


Figure 9: Gallic acid calibration curve.

The concentration of bioactive compounds in *Eucalyptus sideroxylon* varies significantly across regions, solvents, and subspecies, indicating a strong influence of both environmental conditions and extraction methods on phytochemical profiles. Methanolic extracts consistently yield the highest concentrations of polyphenols and tannins, particularly in Miliana and Blida. For example, white-flowered subspecies in Miliana recorded 263.42 mg GAE/g DW for polyphenols and 648.33 mg GAE/g DW for tannins, highlighting methanol's effectiveness in extracting phenolic compounds. Similar high values appear in Blida, confirming methanol's consistent efficiency. In contrast, flavonoids are entirely absent in methanolic extracts across all sites, suggesting that methanol may be less effective for this class of compounds or that these molecules are not as abundant in methanol-soluble forms in the plant matrix.

Ethanol, while producing slightly lower polyphenol values than methanol, is more effective in extracting flavonoids and still retains strong tannin extraction potential. This is especially evident in Medea and Boughezoul, where the white- and red-flowered subspecies show high tannin concentrations—786.94 and 547.82 mg GAE/g DW respectively. Flavonoid

concentrations peak in Medea, particularly in white-flowered aqueous extracts (604.7), underlining ethanol's broader spectrum in extracting diverse secondary metabolites, especially when regional conditions support their synthesis.

Aqueous extracts show more variability but prove efficient for specific compounds, especially flavonoids in regions like Medea and Blida. For example, Medea's aqueous extract of the white-flowered subspecies reveals high flavonoid content (431.85 mg GAE/g DW), and the red-flowered subspecies shows substantial tannin concentration (832.22 mg GAE/g DW) in Boughezoul. This demonstrates that while water may generally be less efficient than organic solvents, it can outperform them for certain compounds under optimal conditions. Medea stands out across all solvents and subspecies, consistently yielding the highest levels of polyphenols, tannins, and flavonoids, indicating that local environmental factors such as climate, soil composition, and plant maturity might promote stronger secondary metabolite production.

Table 3: Phytochemical composition (Polyphenols, Tannins, Flavonoids) of *Eucalyptus sideroxylon* extracts Across different regions (Miliana, Bouguzoul, Medea, Blida) and solvents, expressed in mg GAE/g DW

Extract	Methanol			Ethanol			Aqueous		
Sub-species	WF-Es	RF-Es	PF-Es	WF-Es	RF-Es	PF-Es	WF-Es	RF-Es	PF-Es
Miliana									
Polyphenol	263.42[a]±10	166.48	/	110.59	199.02	/	263.54	220.14	/
Tannin	648.33	564.39	/	474.31	421.36	/	244.36	383.8	/
Flavonoid	/	/	/	/	/	/	/	/	/
Bouguezoul									
Polyphenol	251.99	359.90	/	159.48	150.96	/	281.97	317.09	/
Tannin	469.4	415.83	/	786.94	547.82	/	331.34	832.22	/
Flavonoid	70.72	38.52	/	69.5	33.21	/	48.34	90.2	/
Médéa									
Polyphenol	423.84	380.2	42.93	358.74	421.97	127.74	417.3	474.59	103.36
Tannin	405.34	245.19	203.22	334.1	574.88	604.7	431.85	216.47	489.28
Flavonoid	84.81	80.69	103.42	86.43	52.17	56.4	59.82	36.46	28.34
Blida									
Polyphenol	373.79	528.48	/	272.69	429.67	/	300.77	525.33	/
Tannin	186.1	334.65	/	83.9	258.44	/	225.31	232.49	/
Flavonoid	60.43	64.69	/	40.69	38.84	/	35.88	36.46	/

In terms of subspecies, the white-flowered form generally contains higher concentrations of polyphenols and tannins, particularly in methanolic and ethanolic extracts. The red-flowered subspecies, while sometimes lower in these two compounds, is notably richer in flavonoids, especially in aqueous extracts. The pink-flowered variant, though less represented, shows

significant values in Medea, particularly for flavonoids and polyphenols, suggesting its potential as a valuable chemotype in specific environments. Overall, these findings confirm that both the choice of solvent and regional origin are crucial in determining the phytochemical yield of *Eucalyptus sideroxylon*, with methanol being the most suitable for tannins and polyphenols, ethanol offering a more balanced extraction including flavonoids, and aqueous extracts providing promising results under the right regional conditions. This was done in comparison to the results of ANOVA multivariable.

The current findings on *Eucalyptus sideroxylon* extracts, particularly the high tannin and polyphenol concentrations observed in methanolic and ethanolic extracts from regions like Medea and Blida, are consistent with previous studies but also highlight significant differences based on species, solvents, and geographic origin. For instance, Aid et al. (2023) reported comparatively low flavonoid and tannin levels in aqueous extracts of *E. globulus* leaves. In contrast, our aqueous extracts—especially from Medea—show much higher values, indicating that *E. sideroxylon* may have a richer phytochemical profile or respond better to environmental conditions in the tested regions.

Similarly, Ismahane et al. (2019) found high polyphenol and flavonoid contents in acetate extracts of *E. globulus*, but much lower levels in chloroform extracts. Our data show that methanol and ethanol extracts of *E. sideroxylon* are comparable to the acetate extract in terms of polyphenol and tannin content, reaffirming the role of solvent polarity in efficient extraction.

Hayat et al. (2020) reported a wide range of polyphenol, flavonoid, and tannin concentrations in *Marrubium vulgare*, some overlapping with our findings, especially for tannins. However, *E. sideroxylon* extracts—particularly ethanol-based—demonstrate even higher tannin levels in some samples, suggesting that it may be a more potent source of certain secondary metabolites. These brief comparisons reinforce the phytochemical richness of *E. sideroxylon* and the significant impact of species differences, regional environments, and solvent selection on bioactive compound extraction.

III .2-The study of biological activity

III.2.1-Anti-diabetic activities

The results of the antidiabetic activity of the various methanolic, ethanolic and aqueous extracts are illustrated in figure 10.

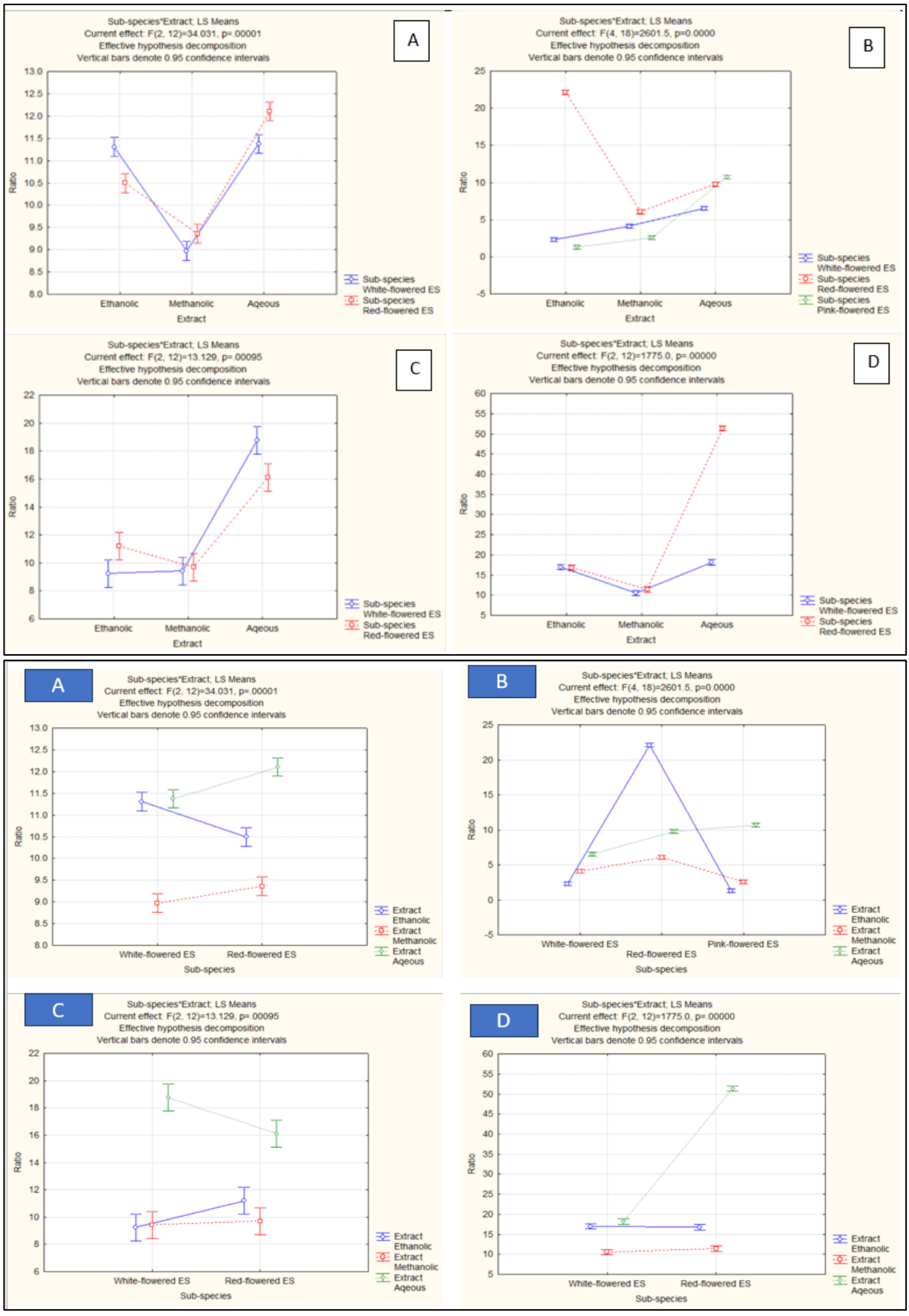


Figure 10: Evaluation of Anti-diabetic activities in *Eucalyptus sideroxylon* sub-species extracts from different regions (A=Bouguezoul; B=Medea; C=Miliana; D=Blida).

The study investigated the anti-diabetic activity of *Eucalyptus sideroxylon* by evaluating the IC50 values (concentration required to inhibit 50% of enzymatic activity) of various extracts (ethanolic, methanolic, and aqueous) from different subspecies (white-, red-, and pink-flowered) collected across four Algerian regions: Médéa, Blida, Miliana, and Bouguezoul. A lower IC50 value reflects a stronger antidiabetic effect.

In the Médéa region, results clearly showed that the pink-flowered ethanolic extract had the strongest anti-diabetic activity, with a remarkably low IC50 of 1.30, forming its own distinct homogeneous group. It was significantly more potent than all other extracts. The white-flowered ethanolic and methanolic extracts followed with moderate activity (IC50 = 2.30 and 2.55 respectively), while aqueous extracts and the red-flowered ethanolic extract performed poorly, with the latter showing the highest IC50 of 22.13, indicating weak antidiabetic potential. This region demonstrated the greatest variation and most significant differences across extracts and subspecies.

In contrast, the Blida region presented overall higher IC50 values, with the white-flowered methanolic extract showing the best activity (IC50 = 10.54), grouped in the least significant category. The red-flowered aqueous extract performed very poorly (IC50 = 51.33), forming its own group "a", indicating a statistically weaker effect. The LSD analysis confirmed substantial differences among extract types, with methanolic extracts outperforming aqueous and ethanolic ones.

The Miliana region showed a narrower range of IC50 values, and the subspecies effect was not statistically significant ($p = 0.70$), suggesting that extract type played a larger role in anti-diabetic activity than flower color. The best performing extract was the white-flowered ethanolic extract (IC50 = 9.24), followed closely by other methanolic and ethanolic extracts. However, aqueous extracts remained significantly weaker (IC50 up to 18.75). Overall, this region had moderate activity with less extreme variability, as reflected by fewer homogeneous LSD groups.

In the Bouguezoul region, all extracts displayed relatively close IC50 values ranging from 8.97 to 12.10. The white-flowered methanolic extract again stood out with the lowest IC50 (8.97), although differences between the best and worst extracts were less pronounced than in other regions. Statistical analysis indicated that extract type remained a significant factor ($p < 0.0001$), while the subspecies had no significant influence ($p = 0.22$), which is consistent with

the findings observed in the Miliana region. Methanolic extracts consistently outperformed aqueous ones.

When comparing all four regions, Médéa clearly stood out due to the superior performance of its pink-flowered ethanolic extract, which was significantly more potent than any other extract across all regions. This result suggests a potentially unique phytochemical profile in this subspecies from Médéa, possibly due to environmental or genetic factors. In general, methanolic and ethanolic extracts consistently demonstrated better antidiabetic activity than aqueous ones, likely due to their enhanced ability to extract bioactive compounds such as polyphenols and flavonoids. Aqueous extracts, in contrast, were repeatedly associated with weaker activity and higher IC50 values.

The current study underscores the importance of both plant origin and extraction solvent in determining anti-diabetic efficacy. The best extract was the ethanolic extract of pink-flowered *Eucalyptus sideroxylon* from Médéa, followed by methanolic and ethanolic extracts of white-flowered subspecies from the other regions. These findings support the use of organic solvents in extraction processes and highlight the medicinal potential of specific subspecies grown in specific regions.

Table 4: Km and Vm results for eucalyptus sideroxylon extract and control for the medea zerouek region.

	Km (µmol/l)	**Vm (µmol/min)**
Ethanol	1,17 ±0.01	0,25 ±0.03
Methanol	1,60 ±0.01	0,37 ±0.02
Aqueous	2,46 ±0.05	0,70 ±0.02
Control	4,08 ±0.03	1,29 ±0.01

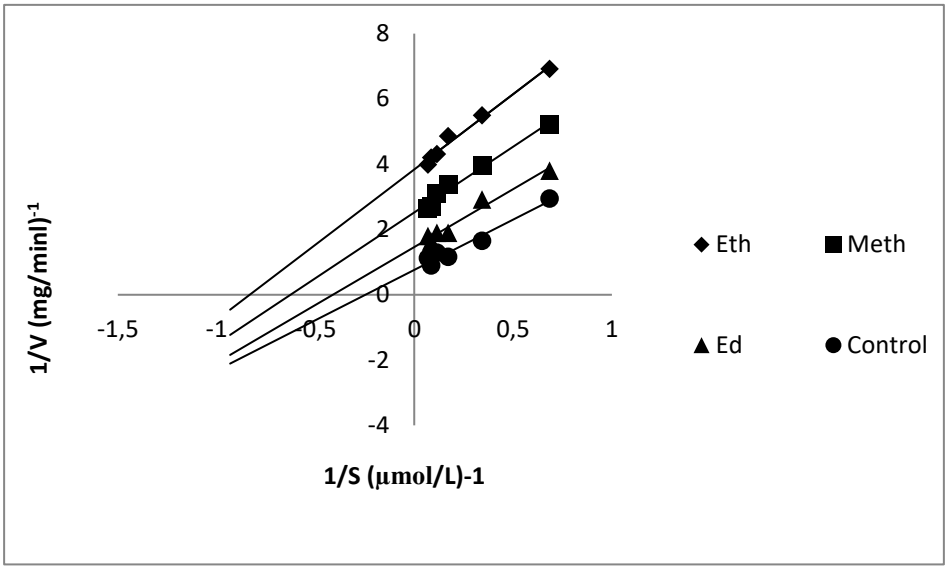


Figure 11: Line weaver-Burk presentation of the different extracts with the control.

The type of inhibition was determined specifically for the Medea zerouek PF region. As shown in Figure 11, the inhibition observed for this region was identified as **uncompetitive**.

Table 7 presents the Km and Vm values for different plant extracts (Ethanol, Methanol, and Aqueous) and a Control. These are key kinetic parameters derived from the Michaelis–Menten equation and help assess enzyme activity and substrate affinity in the presence of each extract. The results demonstrate that the ethanol extract exhibited the highest enzyme affinity and strongest anti-diabetic activity, with the lowest Km (1.17 µmol/L) and Vm (0.25 µmol/min), outperforming both methanol (Km = 1.60 µmol/L, Vm = 0.37 µmol/min) and aqueous extracts (Km = 2.46 µmol/L, Vm = 0.70 µmol/min). All extracts showed significant inhibitory effects compared to the control (Km = 4.08 µmol/L, Vm = 1.29 µmol/min), which had the lowest enzyme affinity.

These findings suggest that the extracts, particularly the ethanol extract, enhance enzyme-substrate interaction and exhibit stronger inhibitory effects. The identification of uncompetitive inhibition in the most active region (Medea zerouek PF) further highlights the potential of this extract in anti-diabetic applications and underlines the importance of solvent selection in optimizing biological activity.

These findings are further supported by previous research. According to Sabiu & Ashafa (2016), the ethanolic extract of *Eucalyptus obliqua* showed a potent inhibitory effect on α-amylase at low concentrations, comparable to acarbose, a standard anti-diabetic drug. However, at higher concentrations, the extract's effectiveness dropped, and its IC50 rose to 2.19 mg/mL, higher than that of the control (0.99 mg/mL), indicating a concentration-dependent inhibition. Similarly, Basak et al. (2010) found that the essential oil of *E. camaldulensis* inhibited both α-amylase and α-glucosidase non-competitively, with an impressive IC50 of 0.435 µl/mL, emphasizing that specific *Eucalyptus* species and compounds can strongly inhibit carbohydrate-digesting enzymes.

Moreover, a recent study by Barakat et al. (2024) provides additional context. Their work reported that the Bouguezoul white-flowered extract achieved the highest α-amylase inhibition (98.9%), consistent with our finding of an IC50 of 8.97 for the same extract. Meanwhile, the Médéa red-flowered extract showed a substantial 76% inhibition, aligning with its moderate IC50 of 6.05. On the other hand, the lowest inhibition percentages were observed for Bouguezoul red-flowered (34%) and Médéa white-flowered (9.96%) extracts, which correlates with their higher IC50 values (Bouguezoul RF aqueous: 12.10; Médéa WF aqueous: 6.51).

III.2.2 -Anti- inflammatory

III.2.2.1- Hypotonic solution-induced hemolysis

In our study, we assessed the anti-inflammatory potential of our *E Sideroxylon* extracts, using the in vitro human red blood cell membrane stabilization test after induction of hemolysis by a hypotonic solution combined with elevated temperature.We used diclophenac as a reference anti-inflammatory agent. The results obtained are summarized in the following graphs.

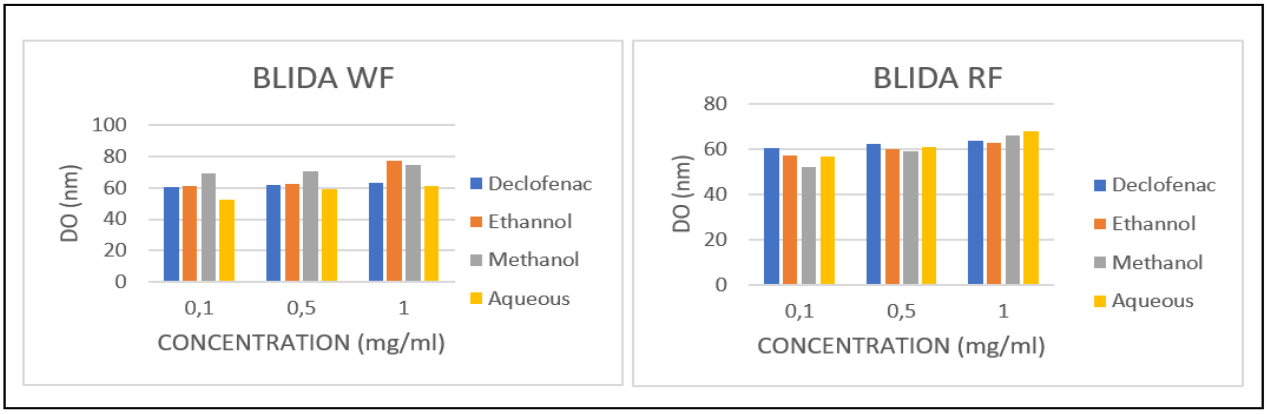


Figure 12: The effect of different concentrations of red and white flowers of *Eucalyptus sideroxylon* in different solvents (ethanolic, methanolic and aqueous) on anti-inflammatory activity in the region of Blida.

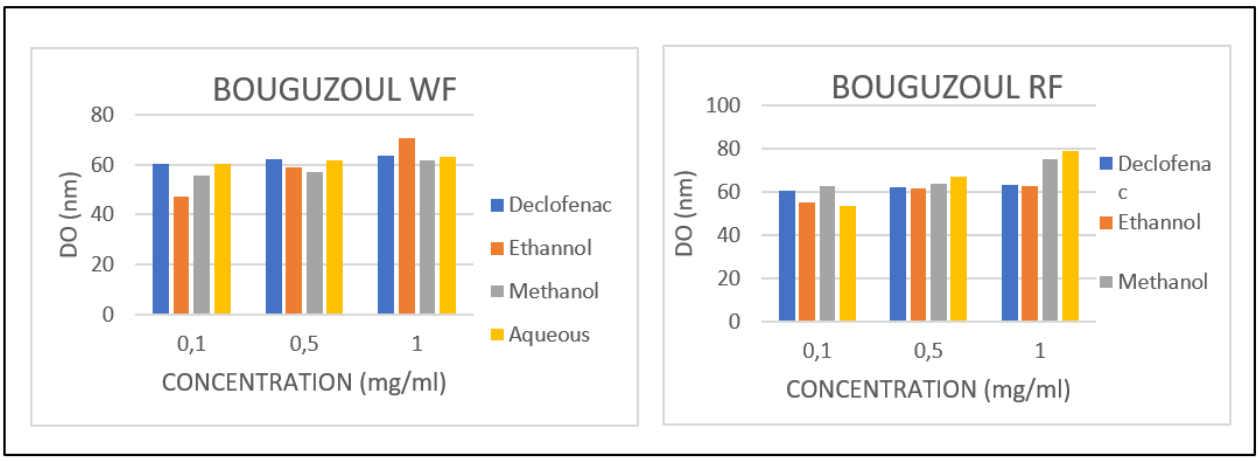


Figure 13: The effect of different concentrations of red and white flowers of *Eucalyptus sideroxylon* in different solvents (ethanolic, methanolic and aqueous) on anti-inflammatory activity in the region of Bouguzoul.

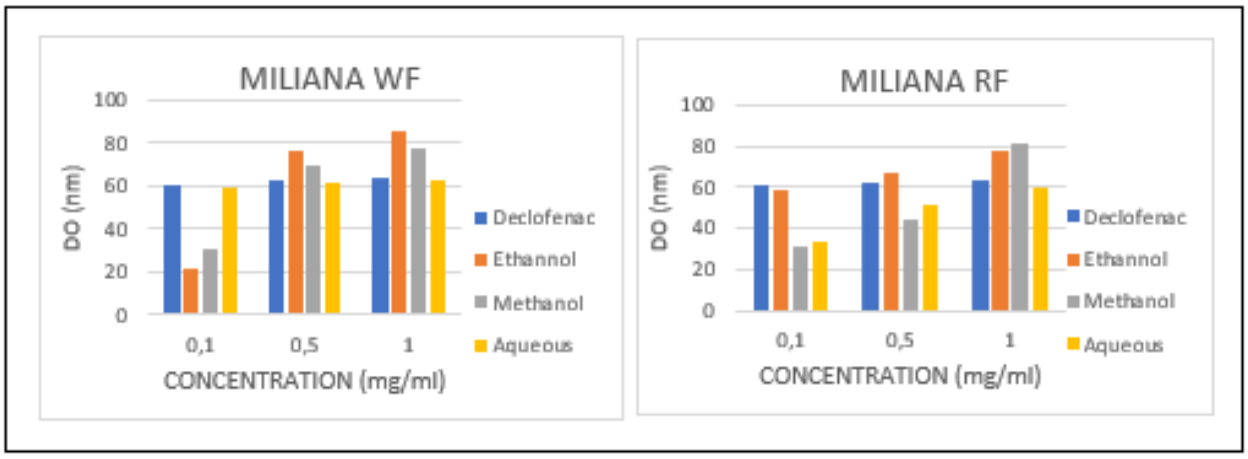


Figure14: The effect of different concentrations of red and white flowers of *Eucalyptus sideroxylon* in different solvents (ethanolic, methanolic and aqueous) on anti-inflammatory activity in the region of Miliana.

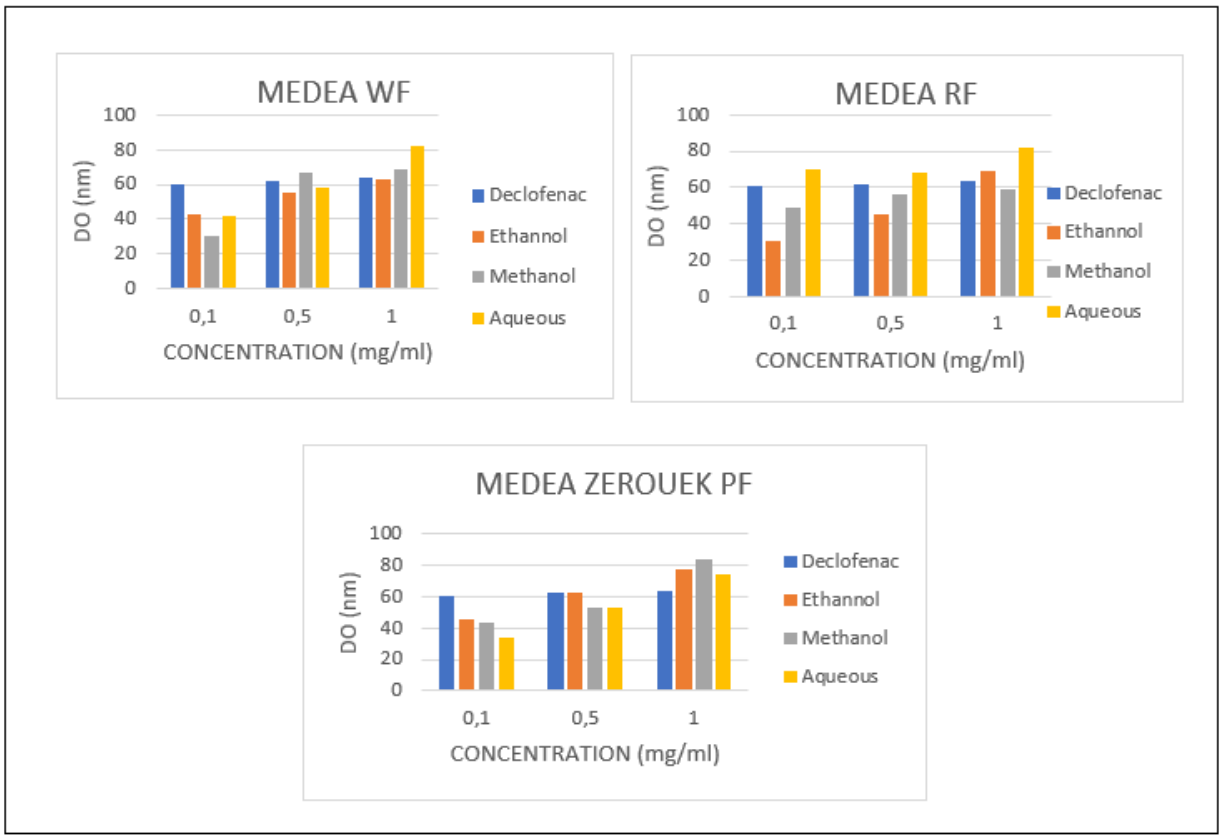


Figure 15: The effect of different concentrations of red, pink and white flowers of *Eucalyptus sideroxylon* in different solvents (ethanolic, methanolic and aqueous) on anti-inflammatory activity in the region of Medea.

The graphs show the result of percentage inhibition of hypotonic solution-induced hemolysis of erythrocyte membrane by the leaf extract of *Eucalyptus sideroxylon* in comparison with that of the reference drug diclophenac.

Methanol consistently produced extracts with moderate anti-inflammatory activity across all regions. In particular: Miliana WF and Medea Zerouek PF methanolic extracts showed relatively high absorption values at 1 mg/ml, indicating enhanced activity with increased concentration.

Ethanol extracts showed moderate to low activity in all tested regions: in Bouguzoul, Blida, and Medea, ethanolic extracts maintained relatively high absorption values across concentrations, they were less effective than methanolic and aqueous counterparts in some cases (e.g., Bouguzoul RF and Medea WF at 0,5 and 1 mg/ml). Across the board, ethanolic extracts were clearly less effective than diclofenac.

Aqueous extracts showed variable results, but in several cases, they demonstrated comparable or slightly better activity than ethanolic extracts: in Medea WF/RF and Blida RF, aqueous extracts at 1 mg/ml showed notable activity, with absorption values approaching those of diclofenac.However, aqueous extracts generally lagged behind methanolic extracts, especially in Medea zerouek PF and Blida WF.While their activity improved with concentration, they still did not match diclofenac's potency except in Bouguzoul ,Medea all species and Blida RF.

III.2.2.2- Heat-induced hemolysis

Table 5: The effect of different concentrations of red, pink and white flowers of *Eucalyptus sideroxylon* in different solvents (ethanolic, methanolic and aqueous) on anti-inflammatory activity in various regions (Miliana, Blida, Medea, Bouguzoul).

Miliana						
Extract	Ethanolic		Methanolic		Aqueous	
Sup species	WF	RF	WF	RF	WF	RF
C1	37.37	11.26	26.75	15.4	56.54	13.12
C2	71.48	16.21	53.39	16.21	60.54	14.23
C3	86.91	24.65	66.22	28.04	78.98	14.75
Bouguzoul						
C1	25.59	28.07	22.61	16.33	32.55	32.94
C2	32.9	32.45	27.71	30.02	31.83	35.08
C3	33.42	34.97	50.38	42.14	40.57	26.69
Medea						
C1	12.01	13.31	12.93	20.33	25.59	25.8
C2	13.58	18.56	14.38	18.48	33	26.83
C3	15.09	25.5	17.7	31	34.14	28.91
Blida						
C1	23.17	14.29	31.64	14.41	28.04	38.38
C2	24.36	23.09	36.03	21.71	32.61	48.77

C3	27.22	39.14	42.02	23.97	33.5	62.63
Medea Zerouek						
C1	/	7.48	/	15.99	/	12.08
C2	/	24.9	/	31.35	/	12.87
C3	/	34.88	/	43.41	/	20.94

Table 6: the effect of different concentrations of diclofenac.

Concentration	C1	C2	C3
Declofenac	60.41	62.11	63.52

The table 5 and 6 presents results of anti-inflammatory activity using the **Fisher LSD (Least Significant Difference) test**, comparing **mean percentage inhibition** of different **plant extracts** (ethanolic, methanolic, aqueous) at various **concentrations (C1, C2, C3)** across multiple Algerian regions (Medea, Bouguizoul, Miliana, Blida). **Diclofenac** is used as a reference anti-inflammatory agent. Methanolic extracts consistently showed the highest and most reliable activity among the plant-based treatments. For instance, methanolic extracts at the highest concentration (C3) reached 50.38% inhibition in Bouguizoul WF, 45.65% in Blida WF, and 66.22% in Miliana WF.

Aqueous extracts demonstrated moderate and region-dependent activity. In some cases such as Blida WF (33.50%) and Bouguizoul WF (40.57%) at C3 they performed comparably to methanolic extracts. However, in other regions and parts like Medea PF and Blida WF, aqueous extract performance was weaker, suggesting that water may extract only certain polar compounds, with limited bioactivity compared to methanol.

Ethanolic extracts, while generally the least effective across most regions, showed a remarkable exception in Miliana WF, where the ethanolic extract at C3 achieved 86.91% inhibition, exceeding even diclofenac. This suggests that ethanol extracted specific potent compounds in this region's plant material that may not be present or as active in other areas. In all other regions, ethanolic extracts showed lower inhibition rates, often below 40%, indicating that ethanol was not as efficient in extracting the active constituents as methanol.

Methanol was the most effective and consistent solvent overall, followed by aqueous extracts, while ethanol was generally the least effective with the notable and exceptional case of Miliana WF, where its performance surpassed all others, including the reference drug diclofenac. These differences emphasize the importance of both solvent choice and plant origin in determining the therapeutic potential of natural extracts.

The extraction of anti-inflammatory compounds from *Eucalyptus sideroxylon* was performed using methanol, ethanol and water. The efficiency of methanol was found to be intermediate, due to its polarity, which solubilises a variety of bioactive constituents, including flavonoids, tannins and phenolic acids. This resulted in higher yields and more consistent bioactivity across regions (e.g. Miliana WF and Medea Zerouek PF), as evidenced by increased optical density values. While ethanol was effective, it extracted fewer polar compounds. Water's limitations in solubilising non-polar metabolites led to variable and often weaker activity. Thus, methanol's balanced polarity and universal efficacy make it the optimal solvent for maximising anti-inflammatory potential in phytochemical extracts.

In the membrane stabilisation assay*, E. sideroxylon bark* exhibited significant ($P < 0.01$) human red blood cell membrane stabilising activity at all of the tested doses (62.5–500 µg/mL). Furthermore, it was superior to the reference drug at all tested concentrations. It is interesting to point out that membrane stabilization was 26% at the lowest tested dose (62.5 µg/mL). Doubling the E. sideroxylon bark dose to 125 µg/mL increased the activity to 34.40%. This is equal to the effect observed by BE at a dose of 250 µg/mL. Doubling the BE dose again (to 500 µg/mL) increased membrane stabilization to 35%. It is not necessary to increase the BE dose from 125 to 500 µg/mL to achieve such a potent effect. **Ashour et al. (2019)** and **Hadj et al. (2021)** have shown that *Bunium incrassatum* extracts (mainly the aqueous extract) have anti-inflammatory effect similar to that of salicylic acid, making it possible to use *Bunium incrassatum* as an alternative treatment to prevent inflammation. At a concentration of 1000 µg/ml, the aqueous extract gave the highest hemolysis inhibition rate (54.38%), followed by the acetone extract (50.87%), while the lowest inhibition rate (47.36%) was observed in the ethanolic extract.

Last year's results showed that all extracts showed significant stabilization of the red cell membrane, with stabilization percentages ranging from 97.58% to 99.15%. The highest percentage of stability was obtained with potassium diclofenac (99.15%), followed by Medea pink flower extracts (98.83%) **(Barakat et al., 2024).**

The methanol and aqueous extracts have shown a moderate to strong concentration-dependent anti-inflammatory activity concerning standard control (Diclofenac Sodium; IC50 116.4µg/ml). Interestingly Methanol bark extract exhibited potent anti-inflammatory activity compared to other extracts (IC50 293.0µg/ml). Methanol and aqueous extracts showed a statistically significant correlation between concentration and percentage inhibition ($p<0.05$, $R2 \approx 1$) **(Hkk et al., 2024).**

Conclusion

The species *Eucalyptus sideroxylon* is known for its significant medicinal properties and is widely used in traditional medicine. The primary aim of this study was to analyze the chemical composition of ethanolic, methanolic, and aqueous extracts derived from red- and white-flowered *Eucalyptus sideroxylon* collected from various regions in Algeria, and to evaluate their biological activities, specifically their anti-diabetic and anti-inflammatory potential.

Our findings revealed that extraction yields varied considerably by region and solvent, ranging from 2.93% to 39.61%, with the highest yield obtained from the ethanolic extract of the red-flowered Bouguezoul sample. Phytochemical screening confirmed the presence of key secondary metabolites such as flavonoids, tannins, saponins, coumarins, cardiac glycosides, and free quinones, while alkaloids, sugars, and proteins were absent.

Quantitative analysis highlighted regional and solvent-related variability in compound concentrations. The red-flowered Blida sample exhibited the highest polyphenol content (528.48 mg GAE/g DW) in the methanolic extract , whereas the lowest was recorded in the ethanolic extract from Médéa-Zerouek (42.93 mg GAE/g DW). Regarding tannins, the aqueous extract from Bouguezoul showed the highest level (832.22 mg EAT/g DW), while the ethanolic extract from Blida contained the least (83.9 mg EAT/g DW). Interestingly, flavonoid content peaked in the ethanolic extract of Médéa-Zerouek (103.42 mg RE/g) and was lowest in aqueous extracts (28.34 mg RE/g).

The evaluation of anti-diabetic activity demonstrated that the extracts exhibited notable inhibitory effects on α-amylase, suggesting a promising potential for managing diabetes. The ethanolic extracts, particularly those from Medea PF and WF, showed the most pronounced effects. In terms of anti-inflammatory activity, all extracts—along with the reference drug diclofenac—showed increasing erythrocyte membrane stabilization with concentration. Methanolic extracts were consistently the most effective, followed by aqueous extracts. Ethanolic extracts were generally less effective, with the exception of the white-flowered sample from Miliana, which showed notable anti-inflammatory activity.

In conclusion, *Eucalyptus sideroxylon* from Algeria presents a rich source of bioactive compounds with significant antidiabetic and anti-inflammatory potential. These promising results warrant further in-depth investigations to isolate and characterize its active constituents, understand their mechanisms of action, and explore their therapeutic potential through experimental and clinical studies.

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ANNEX

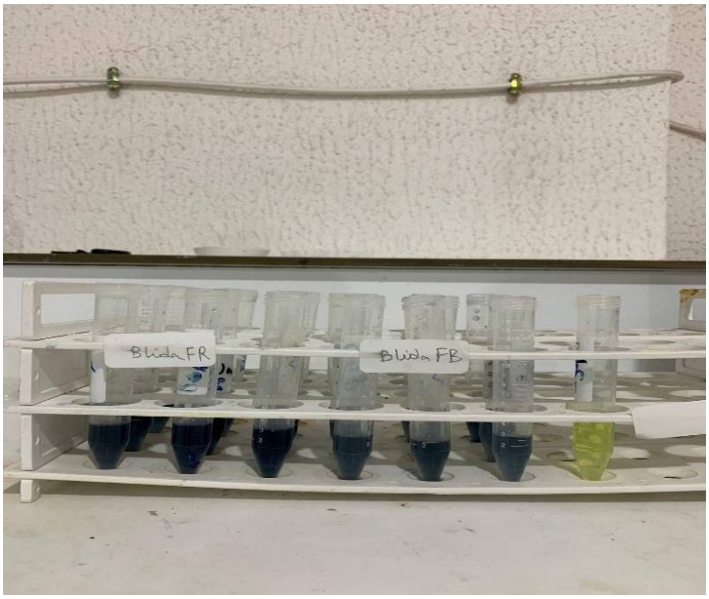




Figure1: determination of total polyphenols





Figure2: dosage of condensed tannins.

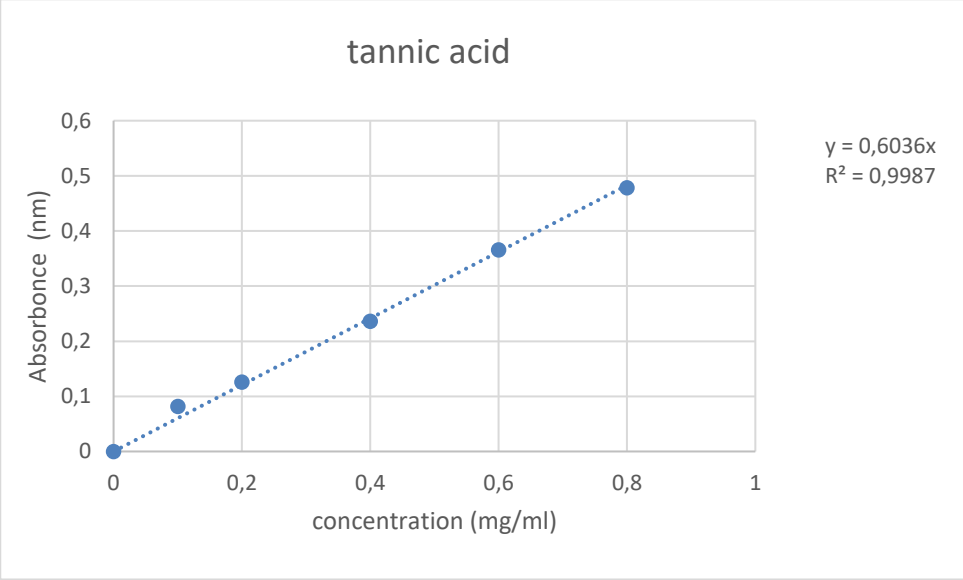


Figure3 : tannic acid calibration curve.

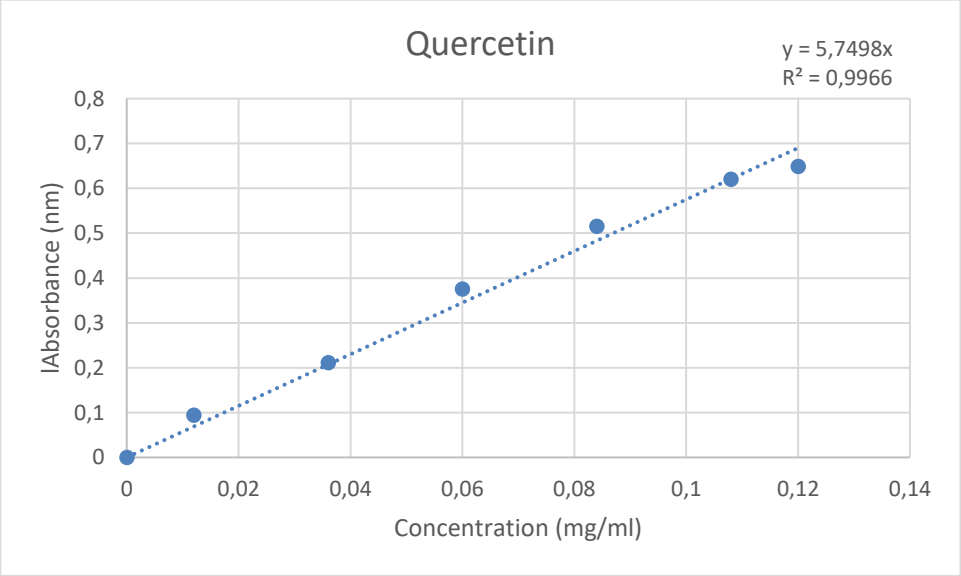
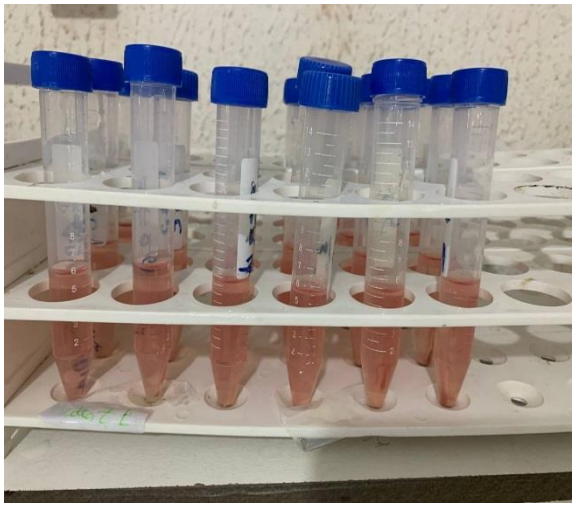
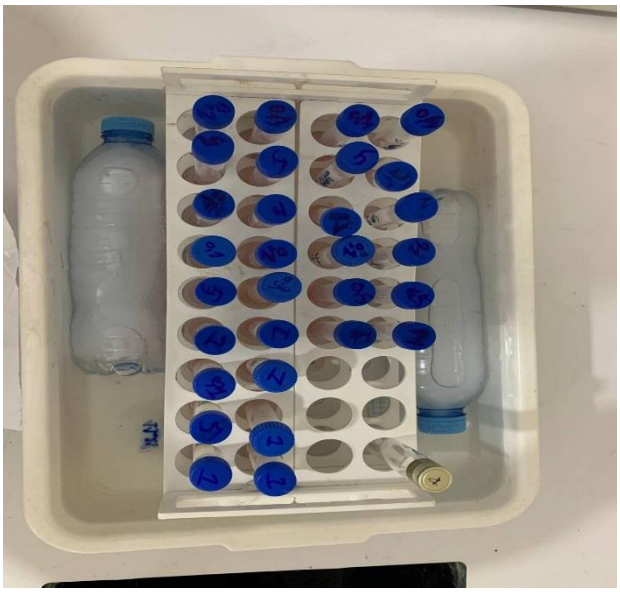


Figure4 : Quercetin calibration curve.





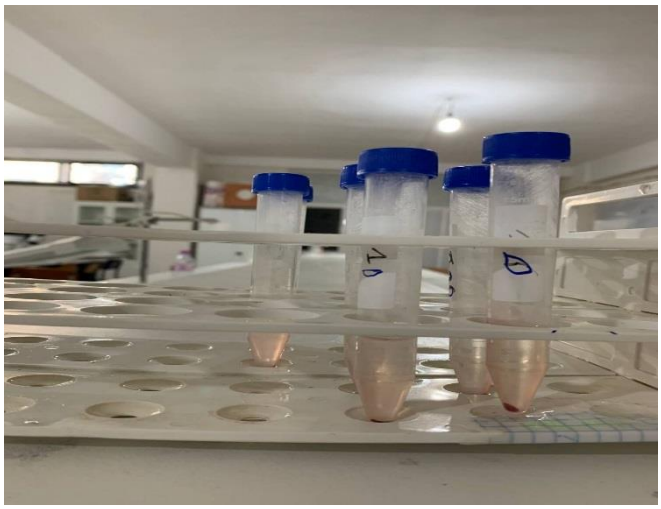


Figure5 : Protocol of anti-inflammatory activity.

Data analysis of the antidiabetic activity

Sub-species	Extract	Ratio	Homogeneous group (HG)
Pink-flowered ES	Ethanolic	1.30000	H
White-flowered ES	Ethanolic	2.30000	G
Pink-flowered ES	Methanolic	2.55500	G
White-flowered ES	Methanolic	4.09000	F
Red-flowered ES	Methanolic	6.05000	E
White-flowered ES	Aqeous	6.51000	D
Red-flowered ES	Aqeous	9.75000	C
Pink-flowered ES	Aqeous	10.71500	B
Red-flowered ES	Ethanolic	22.12500	A

Table 10: Mean comparison by Fisher LSD test for the region of Medea

Sub-species	Extract	Ratio	Homogeneous group (HG)
White-flowered ES	Methanolic	10.53500	D
Red-flowered ES	Methanolic	11.39000	D
Red-flowered ES	Ethanolic	16.75500	C
White-flowered ES	Ethanolic	16.97500	C
White-flowered ES	Aqeous	18.12000	B
Red-flowered ES	Aqeous	51.33500	A

Table 11: Mean comparison by Fisher LSD test for the region of Blida

Sub-species	Extract	Ratio	Homogeneous group (HG)

White-flowered ES	Ethanolic	9.24000	d
White-flowered ES	Methanolic	9.44000	d
Red-flowered ES	Methanolic	9.69500	d
Red-flowered ES	Ethanolic	11.19000	c
Red-flowered ES	Aqeous	16.11500	b
White-flowered ES	Aqeous	18.75500	a

Table 12: Mean comparison by Fisher LSD test for the region of Miliana.

Sub-species	Extract	Ratio	Homogeneous group (HG)
White-flowered ES	Methanolic	8.97000	e
Red-flowered ES	Methanolic	9.36000	d
Red-flowered ES	Ethanolic	10.49500	c
White-flowered ES	Ethanolic	11.30500	b
White-flowered ES	Aqeous	11.37500	b
Red-flowered ES	Aqeous	12.10500	a

Table 13: Mean comparison by Fisher LSD test for the region of Bouguezoul.

Data analysis of the anti-inflammatory activity

	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
White F	36	58.07366	30.17000	82.15297	13.41976	23.10816
Red F	36	59.50779	30.02833	82.29462	13.09310	22.00232
Pink F	36	59.3543	32.1530	84.1360	14.32632	24.13694

Table 14: Descriptive statistics (Min and Max values, CV) of the three subspecies for the region of Medea (Test 1).

	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
White F	36	30.37498	11.52416	63.59773	19.92471	65.59577
Red F	36	32.90074	12.69231	63.59773	17.73469	53.90361
Pink F	36	32.49956	5.676856	63.59773	19.96625	61.43544

Table 15: Descriptive statistics (Min and Max values, CV) of the three subspecies for the region of Medea (Test 2).

	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
White F	36	64.58924	52.26629	77.33711	6.843364	10.59521
Red F	36	60.67044	51.84136	68.55524	4.140175	6.824039

Table 16: Descriptive statistics (Min and Max values, CV) of the three subspecies for the region of Blida (Test 1).

	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
White F	36	38.72540	22.91667	63.59773	14.52854	37.51683
Red F	36	39.37276	11.92308	64.15094	19.20417	48.77527

Table 17: Descriptive statistics (Min and Max values, CV) of the three subspecies for the region of Medea (Test 2).

Extract	Concentration	Inhibition%	Homogeneous group (HG)

Ethanolic	C1	47.30878	H
Methanolic	C1	55.66572	G
Methanolic	C2	57.15297	F
Ethanolic	C2	58.92351	E
declofenac	C1	60.41076	D
Aqeous	C1	60.55241	D
Aqeous	C2	61.68555	C
Methanolic	C3	61.89802	C
declofenac	C2	62.11048	C
Aqeous	C3	63.38527	B
declofenac	C3	63.52691	B
Ethanolic	C3	70.82153	a

Table 18: Mean comparison by Fisher LSD test for the regions of Bouguezoul WF (test 1).

Extract	Concentration	Inhibition%	Homogeneous group (HG)
Aqeous	C1	53.68272	K
Ethanolic	C1	55.24079	J
declofenac	C1	60.41076	I
Ethanolic	C2	61.68555	H
declofenac	C2	62.11048	gh
Methanolic	C1	62.60623	Ef
Ethanolic	C3	62.74788	Ef
declofenac	C3	63.52691	De
Methanolic	C2	63.95184	D
Aqeous	C2	67.13881	C
Methanolic	C3	75.28329	B
Aqeous	C3	79.17847	a

Table 19: Mean comparison by Fisher LSD test for the regions of Bouguezoul RF (test 1).

Descriptive Statistics (Spreadsheet1)						
	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
Inhibition%	36	60.73536	20.82153	89.23513	17.86603	29.41619

Table 20: Result of descriptive statistics (Spreadsheet1) for the region of Miliana WF (test 1).

Descriptive Statistics (Spreadsheet1)						
	Valid N	Mean	Minimum	Maximum	Std.Dev.	Coef.Var.
Inhibition%	36	57.56020	31.16147	85.83569	15.11209	26.25441

Table 21: Result of descriptive statistics (Spreadsheet1) for the region of Miliana RF (test 1).

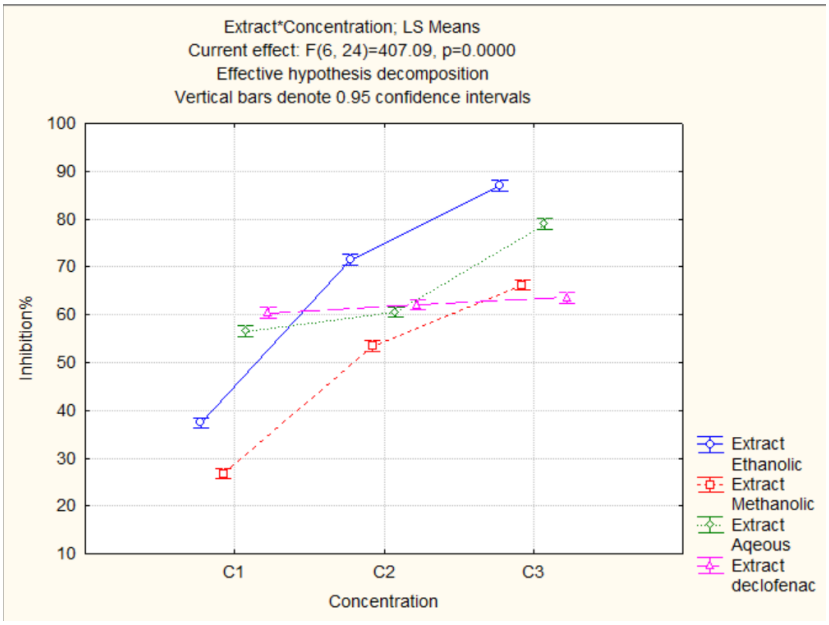


Figure 5: Comparison of anti-inflammatory activity of different extracts at varying concentrations for the region of Miliana WF (test 2).

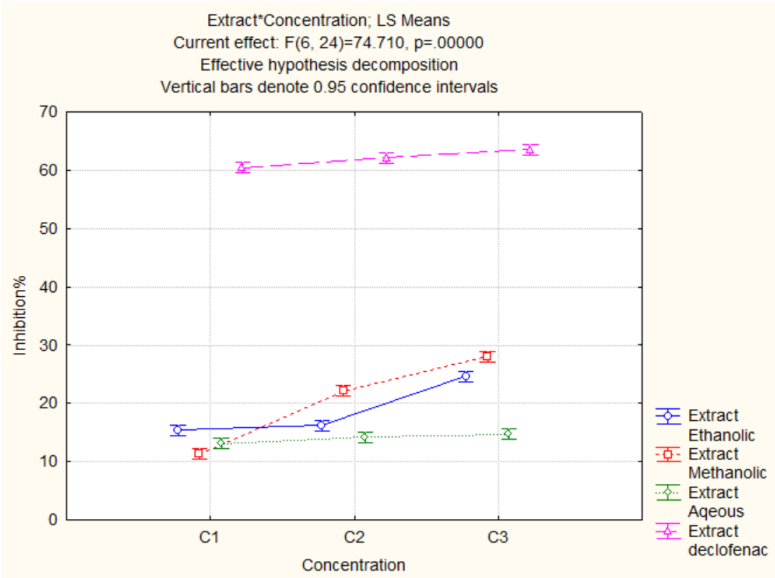


Figure 6: Comparison of anti-inflammatory activity of different extracts at varying concentrations for the region of Miliana RF (test 2).