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## Topic:

# *Biological Activities of the medicinal plant Senna alexandrina: An In vivo, In Vitro, and In Silico Approach*

**Presented by:** **Belkheit Marwa**

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# *Dedication*

*I dedicate this modest work:*

***To my dear family***

*To My mother **Djamila,** for her endless love, unwavering support, and heartfelt prayers.*

*To my beloved father **Abd ELGhani,** for his sacrifices, wisdom, and constant encouragement.*

*To my dearest sister **IMANE**, my second mother, my guardian, my constant light. Your love lifted me, your strength guided me, and your presence gave me the courage to keep going. This work is a reflection of everything you've given me.*

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**Abstract**

This study aims to evaluate the phytochemical composition as well as the antibacterial and gastroprotective activities of the aqueous extract of *Senna alexandrina* leaves. An in *silico* assessment of the natural compounds, conducted through molecular docking simulations, was used to analyze their inhibitory potential on ATPase and polymerase. LC-MS QTOF and FTIR analyses revealed a richness in bioactive compounds, notably polyphenols ($28.42 \pm 0.4$ mg GAE/g), flavonoids ($9.05 \pm 0.84$ mg QE/g), tannins ($0.43 \pm 0.026$ mg CE/g), and the characteristic presence of sennosides, anthraquinone compounds typical of the species. The antibacterial activity was tested against *E. coli (ATCC* 25922*)*, *S. aureus (ATCC* 25923*)*, and *P. aeruginosa (ATCC* 27853*)* at various concentrations (0.3125–200 mg/ml). A dose-dependent effect was observed, with maximum inhibition zones at 200 mg/ml (12 mm for *E. coli*, 10 mm for *S. aureus*, and 9 mm for *P. aeruginosa*). MIC values were 10 mg/ml for *E. coli* and *S. aureus*, and 200 mg/ml for *P. aeruginosa*, with no bactericidal effect, indicating a bacteriostatic action, confirmed by the time-kill assay. The gastroprotective effect was evaluated in mice using ethanol-induced gastric ulcer model (96%, 0.1 ml/mouse). Two doses of the extract (100 and 200 mg/kg) were administered orally (by gavage), and compared to a positive control group treated with omeprazole. The results showed a dose-dependent reduction of gastric lesions, confirmed by a significant decrease in the ulcer index. Histological analysis revealed preserved mucosal integrity and reduced inflammation in treated groups. Molecular docking studies supported these experimental results by confirming potential interactions between the plants major bioactive compounds and various target proteins, notably the bacterial RNA polymerase enzyme and the gastric receptor (ATPASE proton pump). These findings support the results of the study, showing a strong correlation with the experimental data.

**Keywords:** *Senna alexandrina*; Aqueous extract; Antibacterial activity; Time-kill; Gastric ulcer; Gastroprotective effect; Molecular docking.

**Résumé**

Cette étude vise à évaluer la composition phytochimique ainsi que les activités antibactérienne et gastroprotectrice de l'extrait aqueux des feuilles de *Senna alexandrina*. L'évaluation *in silico* des composés naturels, réalisée par des simulations de docking moléculaire, a permis d'analyser leur potentiel inhibiteur sur l'ATPase et la polymérase. Les analyses LC-MS QTOF et FTIR ont révélé une richesse en composés bioactifs, notamment les polyphénols (28,42 ± 0,4 mg EAG/g), les flavonoïdes (9,05 ± 0,84 mg EQ/g), et les tanins (0,43 ± 0,026 mg EC/g), ainsi que la présence caractéristique de sennosides, composés anthraquinoniques typiques de l'espèce. L'activité antibactérienne a été testée contre trois souches de références *E. coli (ATCC* 25922*), S. aureus(ATCC* 25923*)* et *P. aeruginosa (ATCC* 27853*)* à différentes concentrations (0,3125–200 mg/ml). L'effet dose- dépendant, avec des zones d'inhibition maximale à 200 mg/ml (12 mm pour *E. coli*, 10 mm pour *S. aureus* et 9 mm pour *P. aeruginosa*). Les CMI étaient de 10 mg/ml pour *E. coli* et *S. aureus*, et 200 mg/ml pour *P. aeruginosa*, sans effet bactéricide, ce qui indique une activité bactériostatique confirmée par le test de time-kill. L'effet gastroprotecteur a été évalué chez la souris dans un modèle d'ulcère gastrique induit par l'éthanol (96 %, 0,1 ml/souris). Deux doses de l'extrait (100 et 200 mg/kg) ont été administrées par voie orale (gavage), comparée à un groupe témoin positif traité à l'Omeprazole 20mg. Les résultats ont montré une réduction dose-dépendante des lésions gastriques, confirmée par une baisse significative de l'indice ulcératif. L'examen histo-pathologique a révélé une préservation de l'intégrité de la muqueuse et une réduction de l'inflammation chez les groupes traités par l'extrait de la plante étudiée. Les études du docking moléculaire ont soutenu ces résultats expérimentaux en confirmant les interactions potentielles entre les principaux composés bioactifs de la plante et diverses protéines cibles, notamment l'enzyme bactérienne polymérase et le récepteur gastrique (pompe à proton ATPase) impliqué dans la protection de la muqueuse. Les résultats soutiennent les résultats de l'étude d'investigation présentant une action qui a démontré une excellente corrélation avec les résultats expérimentaux.

**Mots-clés :** *Senna alexandrina* ; Extrait aqueux ; Activité antibactérienne ; Time-Kill ; Ulcère gastrique ; Effet gastroprotecteur ; Docking moléculaire.

**ملخص**

تهدف هذه الدراسة إلى تقييم التركيب الكيميائي النباتي وكذلك الأنشطة المضادة للبكتيريا المُحافِظة على الغشاء المخاطي المعدي للمستخلص المائي لأوراق سنا ألكسندرينا. وقد تم تحليل المركبات الطبيعية حاسوبيا (In silico) باستخدام محاكاة الالتحام الجزيئي، من أجل دراسة قدرتها التثبيطية المحتملة على إنزيمات ATPase والبوليميراز. أظهرت تحاليلLC-MS QTOF و FTIR أن المستخلص غنيٌ بالمركبات النشطة بيولوجيًا، لاسيما البوليفينولات( $28,42 \pm 0,4$) ملغ مكافئ حمض الغاليك/غ،(الفلافونويدات $9,05 \pm 0,84$) ملغ مكافئ كيرسيتين/غ، (والتانينات$0,43 \pm 0,026$) ملغ مكافئ كاتيشين/غ بالإضافة إلى الكشف عن مركبات السينوسيدات المميزة من نوع الأنثراكينون، والتي تُعد مركبات نموذجية لهذا النوع النباتي . تم اختبار النشاط المضاد للبكتيريا ضد ثلاث سلالات مرجعية (*E. coli (ATCC* 25922) ، (*S. aureus(ATCC* 25923 و *P. aeruginosa (*ATCC27853) باستخدام تراكيز مختلفة من المستخلص تراوحت بين 0.3125 و200 ملغ/مل. أظهر المستخلص تأثيرًا مرتبطًا بالجرعة، حيث سُجِّلت أكبر مناطق تثبيط عند تركيز 200 ملغ/مل: 12 ملم لـ E. coli، و10 ملم لـ S. aureus، و9 ملم لـ P. aeruginosa. بلغت التركيزات المثبطة الأدنى10 ملغ/مل لكل من E. coli وS. aureus، و200 ملغ/مل لـ P. aeruginosa. لم يُلاحظ أي تأثير قاتل للبكتيريا، مما يدل على أن المستخلص يمتلك نشاطًا مثبط لنمو البكتيري فقط، وقد تم تأكيد ذلك باستخدام اختبار Time-Kill. تم تقييم التأثير الواقي للمعدة لدى الفئران باستخدام نموذج قرحة معدية مستحدثة بالإيثانول (96%، 0.1 مل/فأر). أُعطيت جرعتان من المستخلص (100 و200 ملغ/كلغ) عن طريق الفم، وتمت مقارنتهما بمجموعة إيجابية عولجت بـ أوميبرازول بجرعة 20 ملغ/كغ. أظهرت النتائج انخفاضًا في شدة التقرحات مرتبطًا بالجرعة، وأكدت ذلك انخفاض كبير في مؤشر التقرّح. كما أظهر أظهر الفحص الهيستوباثولوجي (النسيجي المرضي) حفاظًا على سلامة الغشاء المخاطي المعدي وتراجعًا في علامات الالتهاب في المجموعات المعالجة بالمستخلص. دعمت دراسات الالتحام الجزيئي هذه النتائج من خلال تأكيد التفاعلات المحتملة بين المركبات النشطة الرئيسية للنبات ومختلف البروتينات المستهدفة، ولا سيما إنزيم البوليميراز البكتيري ومستقبلات المعدة مضخة البروتونATPase المشاركة في حماية الغشاء المخاطي. تدعم هذه النتائج نتائج الدراسة الاستقصائية التي أظهرت ارتباطًا ممتازًا مع النتائج التجريبية.

الكلمات المفتاحية : سنا ألكسندرينا ، المستخلص المائي، النشاط المضاد للبكتيريا، اختبار الزمن للقتل البكتيري ، قرحة المعدة، التأثير الوقائي المعدي، الالتحام الجزيئي.

# Table of Contents

**List of Figures**

**List of Table**

**List of abbreviations**

| Abbreviation | Full Term |
|---|---|
| **S. alexandrina** | Senna alexandrina |
| **FTIR** | Fourier Transform Infrared Spectroscopy |
| **LC-MS QTOF** | Liquid Chromatography–Mass Spectrometry Quadrupole Time-Of-Flight |
| **MS/MS** | Tandem Mass Spectrometry (Mass Spectrometry/Mass Spectrometry) |
| **amu** | Atomic Mass Unit |
| **CFU** | Colony-Forming Units |
| **MH** | Mueller-Hinton |
| **MIC** | Minimum Inhibitory Concentration |
| **MBC** | Minimum Bactericidal Concentration |
| **NaCl** | Sodium Chloride |
| **$\log_{10}$** | Logarithm base 10 |
| **H&E** | Hematoxylin and eosin (histological stain) |
| **GUI** | Gastric Ulcer Index |
| **GAE** | Gallic Acid Equivalents |
| **QE or QRC** | Quercetin Equivalents |
| **CE or CAT** | Catechin Equivalents |
| **TFC** | Total Flavonoid Content |
| **$R^2$** | Coefficient of determination |
| **S. aureus** | Staphylococcus aureus |
| **E. coli** | Escherichia coli |
| **P. aeruginosa** | Pseudomonas aeruginosa |

| | |
|---|---|
| **$H^+/K^+$-ATPase** | Hydrogen/potassium adenosine triphosphatase (proton pump) |
| **SOD** | Superoxide Dismutase |
| **CAT** | Catalase |
| **GPX** | Glutathione Peroxidase |
| **Nrf2** | Nuclear factor erythroid 2–related factor 2 |
| **$PGE_2$** | Prostaglandin $E_2$ |
| **NF-κB** | Nuclear Factor kappa-light-chain-enhancer of activated B cells |
| **TNF-α** | Tumor Necrosis Factor alpha |
| **IL-1β** | Interleukin 1 beta |
| **IL-6** | Interleukin 6 |
| **NOS2** | Nitric Oxide Synthase 2 |
| **NO** | Nitric Oxide |
| **EGF/EGFR** | Epidermal Growth Factor / Epidermal Growth Factor Receptor |
| **MAPKs** | Mitogen-Activated Protein Kinases |
| **ROS** | Reactive Oxygen Species |
| **DNA** | Deoxyribonucleic Acid |
| **mg/ml** | Milligrams per milliliter |
| **m/z** | Mass-to-Charge Ratio |
| **kV** | Kilovolt |
| **%** | Percent |
| ΔG | Binding affinity (free binding energy) |
| **Ki** | inhibition constant |

# General Introduction

## INTRODUCTION

The use of medicinal plants as a therapeutic foundation is an ancient and widespread practice **(Petrovska, 2012)**. For millennia, human societies have relied on natural resources to prevent and treat various health conditions **(Davis and Choisy, 2024; Hindi *et al*., 2024)**. This tradition, rooted in empirical knowledge passed down through generations, continues to influence both traditional and modern healthcare systems **(Ouma, 2022).**

Medicinal plants, known for their diverse therapeutic properties, have been used to treat a wide range of illnesses, from the mildest to the most severe. Interest in these natural remedies has moved beyond traditional contexts and is now firmly embedded in scientific research **(Jamshidi-Kia et al., 2017; Vardhan and Sahoo, 2020)**

Significant advances in biochemistry and pharmacognosy have enabled researchers to isolate, characterize, and integrate bioactive compounds into modern pharmacopeias. These developments providing robust scientific validation for traditional practices, reinforcing their credibility and paving the way for their integration into contemporary medicine **(Fabricant & Farnsworth, 2001**).

One of the greatest assets of medicinal plants lies in their biochemical diversity, particularly in their secondary metabolites such as flavonoids, alkaloids, tannins, and terpenoids) **(González Mera et al., 2019)**. These compounds exhibit a broad spectrum of pharmacological activities such anti-inflammatory, antioxidant, antimicrobial, and anticancer properties **(Firdous et al., 2025).**

These molecules are responsible for the therapeutic potential of plants and act through various biological mechanisms at the cellular level, offering promising avenues for the treatment of complex diseases **(Elshafie et al., 2023).** Notable examples include artemisinin, extracted from Artemisia annua used in malaria treatment **(Haerdi & Veuthey, n.d.),** and Paclitaxel, extracted from Taxus species, which is a key component in the treatment of several types of cancer **(El Houda, n.d.).** These successes highlight the considerable potential of medicinal plants in modern therapeutics, and underscore the importance of continued research in this filed **(Emeje *et al.,* 2024).**

Investigating these natural compounds contributes to a deeper understanding of molecular mechanisms involved in various pathologies and may lead to more targeted, effective and often less invasive treatments than those based solely on synthetic drugs **(Nasim et al., 2022)**. Moreover, research into medicinal plants aligns with sustainable development strategies,

as it promotes the preservation of local biodiversity and traditional knowledge systems **(Mbelebele *et al.*, 2024).**

Beyond their medicinal value, these plants also hold substantial socio-economic significance, particularly in developing countries **(Khesht *et a*l., 2021).** The collection, cultivation, and commercialization of medicinal plants not only foster local economies but also promote gender equity, as women are often the primary custodians of traditional phytotherapeutic knowledge, as such, this filed supports several sustainable Development Goals (SDGs), notably those related to health and poverty reduction. **(Ndhlovu *et al.*, 2023; Suryavanshi *et al.*, 2020; México *et al.*, 2018).**

In this global context, Algeria stands out for its exceptional floristic diversity, owing to its wide range of climatic and geographic zones from humid Mediterranean coast to arid Saharan desert **(Laouar *et al.*, 2010)**. The country hosts more than 3,000 plant species, around 300 of which are known for their medicinal properties **(Kouider *et al.*, 2019)**. However, despite this considerable potential, many of these species remain underexplored from a scientific standpoint, especially in terms of their specific biological activities and detailed phytochemical profiles **(Bouzabata, 2017).**

Among these species of interest, *Senna alexandrina (*also known as Cassia Senna or Cassia angustifolia Vah), a member of the Fabaceae family **(Farag *et al.*, 2015; Chouksey et *al.*, 2024)**, is an annual medicinal plant primarily recognized for its agricultural, industrial, and ethno-medicinal applications **(Dwivedi et al., n.d.)**

Traditionally Senna used as a powerful purgative, it is especially valued for its high content of anthraquinone derivatives, notably sennosides A and B **(Aurapa and Wandee, 2009; El-Hassan *et al.*, 2012).**

Historical records trace its use back to Greco-Arab medicine, and the plant was once widely exported from the port of Alexandrina to Europe and Asia. Even today, it remains a core ingredient in many natural laxative preparations **(Lal *et al.*, 2023).**

From a botanical perspective, *Senna Alexandrina* is a perennial subshrub that typically grows between 60 and 75 cm in height. It is characterized by pinnate compound leaves measuring (2.5−6 cm long and 7−8 mm broad), bright yellow flowers grouped in axillary clusters, and flat pods containing several brownish seeds. The plant is well adapted to harsh

desert environments and grows spontaneously in hot, arid regions **(Lal *et al.*, 2023; Abbas and Rani, 2020)**

Despite the well-documented use of *S. alexandrina* as a natural laxative, its broader therapeutic potential particularly its antibacterial and anti-inflammatory properties remain largely unexplored. In light of rising antibiotic resistance and the need for better tolerated anti-inflammatory agents, medicinal plants represent a promising alternative. It is therefore essential to reevaluate selected species through the lens of new pharmacological properties, using modern experimental methodologies. A combined approach that integrates biological evaluation (in vitro and in vivo) with precise chemical characterization could enable the scientific valorization of this underutilized natural resource.

This study aims to investigate the potential therapeutic properties of this natural resource, with particular focus on its antibacterial and gastroprotective activities. Building on its long-standing use in traditional medicine, the research will provide scientific validation for these applications through detailed chemical characterization and biological evaluation.

To achieve this, the research will:

1-Characterize the chemical composition of *the plant* extract using FTIR spectroscopy and LC-MS q-TOF analysis.

2-Evaluate the antibacterial effect of *S. alexandrina* aqueous extract on various bacterial references strains using *in vitro* assays.

3-Assess its gastroprotective activity using an *in Vivo* animal model.

4-Conduct molecular docking studies to predict interactions between bioactive compounds and receptors.

# Materials and Methods

## Materials and Methods

The aim of this study was to evaluate the biological effects of *Senna alexandrina* using a combined *in vivo, in vitro* and *in silico* approach. The experimental work was conducted in the Biochemistry and Microbiology laboratories, and the animal facility of the University of Guelma (8 May 1945), as well as in the Pathological anatomy and Bacteriology departments of Ibn Zohr Hospital in Guelma.

### I. *In vitro* experiments on *Senna Alexandrina*

#### I-1. Plant material

*Senna alexandrina* is a flowering plant species from the Fabaceae family. It originates from the arid and semi-arid regions of Est Africa and the Arabian Peninsula. While it is predominantly cultivated and harvested in North Africa, including India and Pakistan **(Lal *et al.*, 2023)**. Due to its medicinal properties, it is now widely cultivated in many parts of the world. The following map illustrates its current global distribution **(POWO, n.d.).**

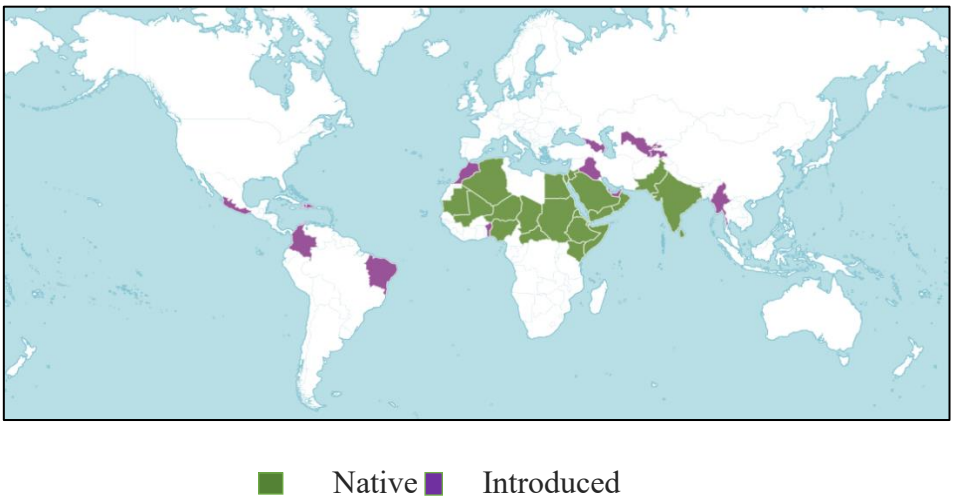


**Figure 1**: Global distribution of *Senna alexandrina* **(POWO, n.d.).**

**Native to:**

Algeria, Central African Republic, Chad, Djibouti, Egypt, Eritrea, Ethiopia, Gulf of Guinea Is., India, Kenya, Mali, Mauritania, Niger, Nigeria, Oman, Pakistan, Palestine, Saudi Arabia, Sinai, Socotra, Somalia, Sri Lanka, Sudan-South Sudan, Yemen

**Introduced into:**

Benin, Brazil Northeast, Colombia, Dominican Republic, Gulf States, Haiti, Iraq, Leeward Is., Mexico Southwest, Morocco, Myanmar, Tadzhikistan, Transcaucasus, Uzbekistan

### I.1.2. Sampling strategy, Collection of the medical plants

The plant leaves under investigation were purchased in May 2024 from Oued Zenati, a locality situated in the commune of Guelma, Algeria. The Identification of the species were carried out with the assistance of a local herbalist from the Guelma region. The samples were collected, meticulously stored in plastic containers and subsequently transported to the Department of Biology, within the Faculty of Natural and Life Sciences, Earth and Universe, for the preparation of leaf extract and comprehensive studies.

### 1.1.3. Preparation of Aqueous Leaf Extract of *Senna alexandrina*

The leaves of this plant were meticulously cleaned, dried, and stored in a dark place to ensure optimal preservation. Subsequently, the leaves were subjected to a process of grinding using an electric grinder, resulting in the formation of a fine powder.

An extraction method based on that by **(Al-Taie, 2019)**, with some modifications. Briefly, 150 g of powdered leaf in 1500ml of distilled water, maintained at room temperature for 24 h. Extract was first filtered on filter paper and lyophilised .

### II.2. Methods

### II.2.1 Extract Characterization:

#### II.2.1.1. LC-MS-qTOF metabolomic analysis

The LC-MS-qTOF (Quadrupole Time-of-Flight Liquid Chromatography–Mass Spectrometry) analysis was employed to perform an untargeted metabolomic screening of the plant extract, aiming to identify and characterize a wide range of secondary metabolites based on their accurate mass and fragmentation patterns **(Allen and McWhinney, 2019).**

The LC/MS QTOF analysis protocol was performed according to the method described by **(Kalyniukova *et al.*, 2024**).

Analysis was conducted using an Agilent 1290 Infinity II system paired with an Agilent 6546 LC/MS QTOF instrument (Agilent, USA). Chromatographic separation was carried out on an Agilent InfinityLab Poroshell 120 EC-C18 column (2.1 x 150 mm, 2.7 µm).

The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol. The gradient profile was as follows: 0–4 minutes at 85% A, 4–7 minutes at 75% A, 7–9 minutes at 68% A, 9–16 minutes at 60% A, 16–22 minutes at 45% A, and 22–28 minutes at 5% A, followed

by 2 minutes at 5% A. The flow rate was maintained at 0.5 mL/min with a column temperature of 35 °C, and 1 µL of sample was injected. Both positive and negative ionization modes were employed. The QTOF parameters included a scan range of 100–1000 m/z, drying gas temperature at 160 °C, sheath gas flow rate at 12.0 L/min, sheath gas temperature at 400 °C, capillary voltage of 5.0 kV, nozzle voltage of 2.0 kV, fragmentor voltage set to 140 V, and collision energies of 10, 20, and 40 eV. For MS/MS analysis, the scan range was 50–800 m/z, with a retention time window of 0.5 minutes, an isolation window of 1.3 amu, and an acquisition rate of 3 spectra per second. Two reference masses (112.9855 m/z and 966.0007 m/z) were used for continuous mass correction. Agilent MassHunter Qualitative Analysis 10.0 was used to proceed the data. For the identification of key metabolites, targeted MS/MS was performed, and the results were compared with the Metline Database, an internal library, and relevant literature based on retention times and MS/MS fragmentation patterns.

#### II.2.1.2. FTIR Analysis (Fourier Transform Infrared Spectroscopy)

Ftir spectroscopy was employed to identify the functional groups and assess the chemical structure of the *Senna alexandrina* extract. This technique provides qualitative information about molecular vibrations and the presence of specific chemical bonds **(Siddique, 2024)**.

Spectral analyses were performed using a Spectrum one spectrometer (Perkin Elmer), operated via a microcomputer running Spectrum Software. Spectra were acquired in the mid-infrared (MIR) range, depending on the selected source, beam splitter, and detector configuration.

Prior to analysis, spectra were preprocessed to enhance signal quality and reduce data complexity. These steps included noise reduction, baseline correction, refinement of absorption bands, and elimination of irrelevant intensity variations. A linear baseline correction was applied to correct sloped baselines, improving the accuracy of spectra affected by sample physical properties and optical path variations.

### II.2.2. Quantification of Phenolic Compounds:

#### II.2.2.1. Polyphenols content determination

The total phenolic content of the extract was determined using the Folin–Ciocalteu colorimetric method **(Li *et al*., 2007)**.

**Principle of the Folin–Ciocalteu Method**

In this method, phenolic compounds reduce the Folin-Ciocalteu reagent under alkaline conditions, leading to the formation of a blue-colored complex **(Malta & Liu, 2014)**. The intensity of this bleu coloration, measured spectrophotometrically at 765 nm, is directly proportional to the concentration of phenolic compounds present in the sample. Quantification is achieved by comparing the absorbance values to a standard calibration curve prepared with gallic acid, and results are expressed as milligrams of gallic acid equivalents per gram of extract **(Ainsworth & Gillespie, 2007)**.

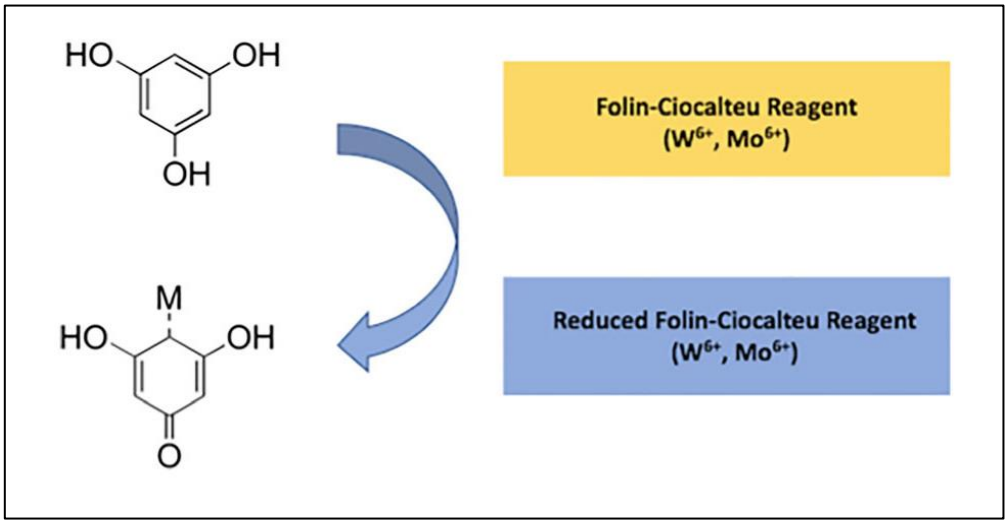


**Figure 2**:Diagram showing the reduction of the Folin–Ciocalteu reagent caused by the oxidation of the phenolics in a sample (**Ford *et al.*, 2019**).

1ml of Folin reagent (diluted 10-fold) is added to 200 µl of either the sample or the standard, prepared with appropriate dilutions. After 4 minutes, 800 µl of a sodium carbonate solution (0.75%) is added to the reaction mixture. The solution is then incubated at room temperature for 2 hours. Absorbance is measured at 765 nm. The total polyphenol content is determined using a calibration curve established with gallic acid (0–250 µg/ml) and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

**II.2.2.2. Flavonoids content determination**

The total flavonoid content in the aqueous extract of S. alexandrina is determined using the method established by (**Bahorun *et al.*, 1996**).

This method is based on the ability of flavonoids to bind to metal ions such as $Al^{3+}$, forming colored complexes. Aluminum reacts with the hydroxyl groups of flavonoids, creating a stable complex whose color intensity ca be measured by spectrophotometry at specific

wavelength typically 430 nm. The intensity of the formed color is proportional to the flavonoid concentration in the extract **(Shraim *et al.*, 2021).**

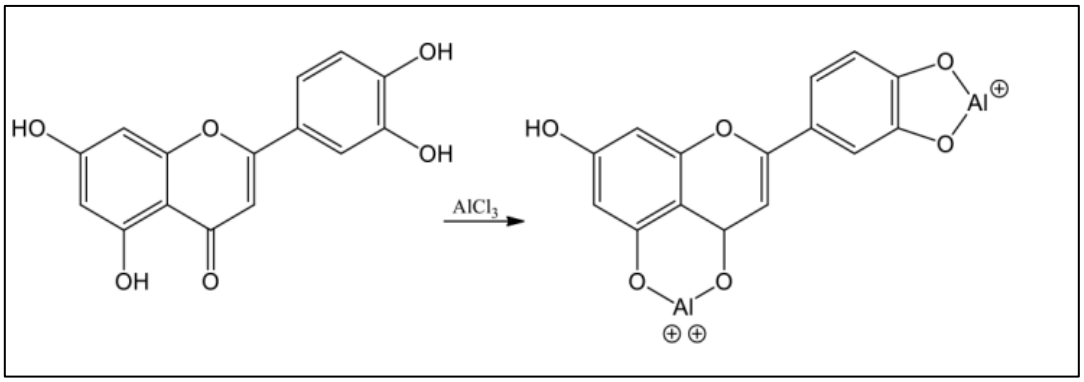


**Figure 3**:The chemical reaction of aluminum chloride method for flavonoids assay (**Tayone *et al.*, 2021)**

Samples are prepared by dissolving 1 mg of extract in 1 ml of water. Then 1 ml of each samples is mixed with 1 ml of aluminum chloride solution. After 10 minutes of reaction, the absorbance is measured at 430 nm.

A standard curve is separately prepared using quercetin (0–40 µg/ml) to calculate the flavonoid concentration in each extract. The results are expressed as milligrams of quercetin equivalents per gram of extract.

### II.2.2.3 Tanins content determination

The condensed tannin content was determined using the method established by **(Julkunen-Titto, 1985).**

The vanillin-HCL colorimetric method for determining condensed tannins is based on a specific chemical reaction between the terminal flavonoid units of proanthocyanidins (condensed tannins) and vanillin in an acidic medium. This reaction produces a red-colored complex, the intensity of which is proportional to the concentration of condensed tannins in the sample **(Sun *et al.*, 1998)**

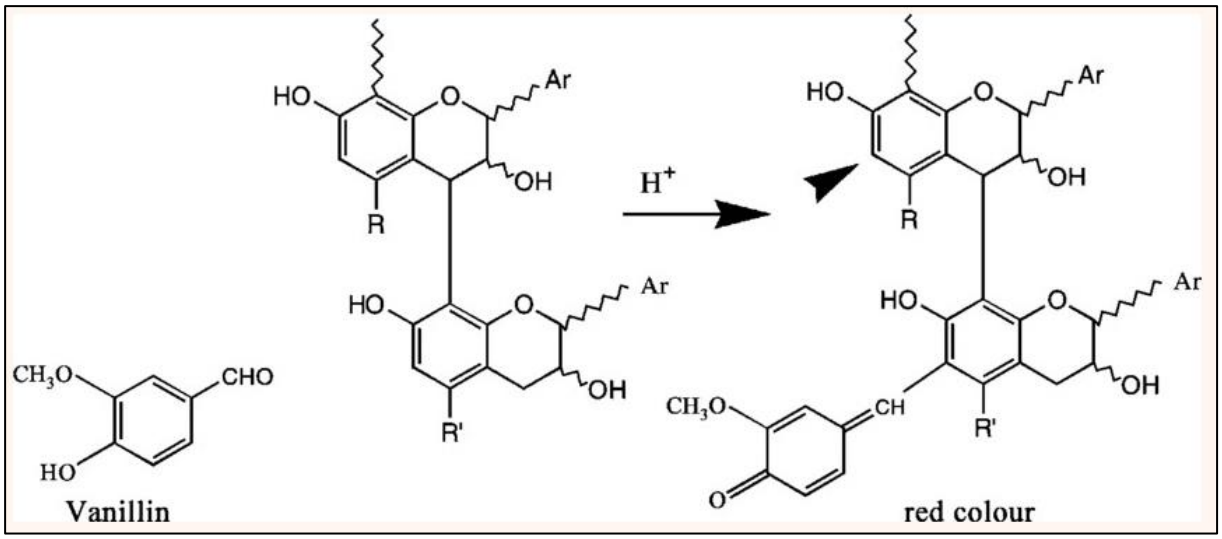


**Figure 4:** Principle of Vanillin HCl Test (**Pharma Academias, 2024**).

A volume of 0,5 ml of the extract was added to 1.5 ml of a 4% vanillin solution. The resulting mixture was vigorously shaken, followed by the addition of 750 µl of concentrated HCL. The reaction mixture was then allowed to stand at room temperature for 2o minutes in a dark chamber. Absorbance was measured at 500 nm against a blank consisting of the 4 % vanillin solution.

**III. Evaluation of Antibacterial Activity**

**III.1. Bacterial strains**

The bacterial strains used in this study were obtained from the Bacteriology laboratory of Ibn Zohr Hospital in Guelma (Algeria). They are internationally recognized as standard reference strains for antibacterial activity testing, according to the recommendations of the American Type Culture Collection (ATCC) and the Clinical and Laboratory Standards Institute (CLSI). The strains were maintained on nutrient agar at 4°C until use **(Basiru et al., 2013)**. and continuously cultured by regular subculturing and replating to ensure their viability for each use. The reference bacteria are: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

**III.2 Determination of antibacterial activity by disk diffusion method**

The antibacterial activity of the aqueous extract was evaluated using the standard disk diffusion method, based on the measurement of inhibition zones **(Saxena *et al.*, 2018).**

Each strain was cultured for 18-24 hours at 37 °C on nutrient agar. Fresh colonies were suspended in sterile physiological saline solution, and the suspension was adjusted to a turbidity

equivalent to the 0.5 McFarland standard (approximately $1\times10^8$ CFU/mL). The optical density was verified using a spectrophotometer at a wavelength of 625 nm to ensure standardization of the inoculum **(Alemu *et al.*, 2024).** Subsequently, the bacterial concentration was diluted to a ratio of 1:100 in a sterile physiological saline solution. This ensured a final bacterial concentration of approximately $1\times10^6$ CFU/mL, which is within the optimal range for subsequent assays **(Kanga and Bolou, 2024).**

The aqueous extract of Senna alexandrina was prepared at various concentrations for antibacterial testing, the procedure was initiated with a stock solution of 200mg/ml, successive dilutions were performed to obtain the following concentrations: 200 mg/mL, 100 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.312 mg/mL. Mueller-Hinton agar were inoculated with 100 µL of the prepared bacterial suspension using a sterile cotton swab to ensure uniform growth. Sterile paper disks (6 mm diameter) were impregnated with 50 µL of the aqueous plant extract at the different concentrations prepared and carefully placed onto the inoculated agar surface **(Bhat & Al-daihan, 2014).** The plates were incubated at 37°C for 18–24 hours. After incubation, the inhibition zones surrounding the disks were examined and measured in millimeters **(Sikrodia *et al.*, 2018).**

The following table summarize the interpretation of bacterial sensitivity **(Mouas *et al.*, 2017).**

**Table 1:** Interpretation of antimicrobial sensitivity

| Sensitivity | Inhibition Zone Diameter |
|---|---|
| **Resistant or non-sensitive (–)** | < 8mm |
| **Sensitive (+)** | 9-14 mm |
| **Very sensitive (++)** | 15-19 mm |
| **Extremely sensitive (+++)** | > 20 mm |

### III.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism under standardized conditions **(Kowalska-Krochmal and Dudek-Wicher, 2021).**

The MIC of the aqueous extract of *Senna Alexandrina* was determined using broth dilution method in test tubes. A total of 10 sterile test tubes were prepared and labeled from C0 to C9. Tube C0 served as the negative control and contained 1 ml of sterile distilled water

without the plant extract. Tubes C1 through C9 each received 1 ml of the plant extract at the following concentrations :0.312, 0.625, 1.25, 2.5, 5, 10, 20, 100, and 200 mg/mL, respectively.

To each tube, 1 ml of a bacterial suspension standardized to 0.5 McFarland turbidity standard was added, resulting in a final volume of 2 ml per tube. The tubes were gently mixed to ensure homogeneity and then incubated at 37°C for 24 hours **(Toty *et al.*, 2013).**

The tubes were examined for turbidity. The MIC was defined as the lowest concentration of the plant extract that completely inhibited visible bacterial growth, indicated by the absence of turbidity in the tube **(Victor and Gaël, 2019).**

### III.4 Determination of Minimum Bactericidal Concentration (MBC)

The MBC is defined as the lowest concentration of an antimicrobial agent required to kill 99.99 % of the initial bacterial population, leaving at most 0.01 % of viable organisms. **(La *et al.*, 2008)**.

Following the MIC determination ,0.1 ml from each tube that showed no visible turbidity was aseptically transferred and streaked in 5 cm parallel lines onto freshly prepared sterile nutrient agar plates, poured into Petri dishes. The inoculated plated were then incubated at 37°C for 24 hours. After incubation, colonies were counted directly to assess bacterial survival. The MBC was identified as the lowest concentration, equal to or higher than the MIC, at which no bacterial colonies were observed **(Ouattara *et al.*, 2013).** All tests were performed in triplicate to ensure accuracy and reproducibility.

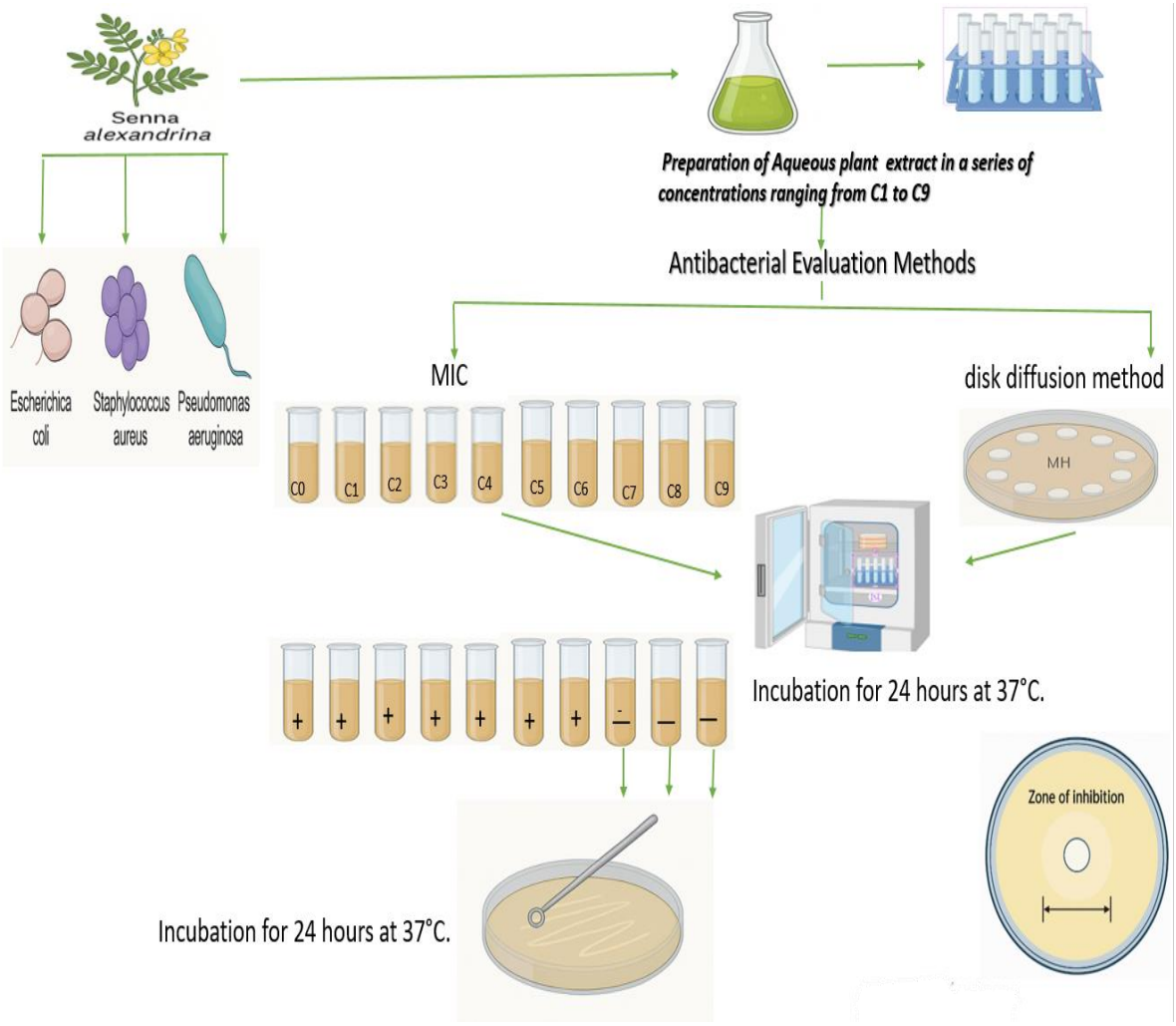


**Figure 5**: Disk diffusion assay and MIC/MBC protocol of *Senna alexandrina* extract.

**III-5 Determination of Time of Kill**

The time kill assay is a widely accepted method for evaluating the bactericidal or fungicidal effects of antimicrobial agents. its provides valuable insights into the dynamic interactions between a compound and a microbial strain, allowing the assessment of whether the antimicrobial activity is time-dependent or concentration-dependent **(Balouiri *et al., 2016)***.

Assays for the rate of killing bacteria by the aqueous extract were carried out using a modified plating technique of **(Eliopoulos and Eliopoulos, 1988 ; Eliopoulos and Moellering, 1996).**The extract was incorporated into 10 mL Mueller Hinton broth in McCartney bottles at ½ MIC, MIC and 2× MIC. Two controls, one Mueller Hinton broth without extract inoculated with test organisms and Mueller Hinton broth incorporated with the extract at the test concentrations without the test organisms, were included. Inoculums density, approximately 105 CFU/mL further verified by total viable count, was used to inoculate 10 mL volumes of both test and control bottles. The bottles were incubated at 37 °C on an orbital shaker at 120

rpm. A 100 µL aliquot was removed from the culture medium at 0,2, 4,6 and 8 h for the determination of CFU/mL by the plate count technique **(Cruishank *et al.*, 1975)** by plating out 25 µL of each of the dilutions. The problem of extract carryover was addressed by dilution as described previously by **(Pankuch *et al.*, 1994)**. After incubating at 37 °C for 24 h, emergent bacterial colonies were counted, CFU/mL calculated and compared with the count of the culture control without extract.

Bactericidal activity was defined as a ≥99.9% (≥3 $\log_{10}$) reduction in the original inoculum 's colony-forming units (CFU) per milliliter. In contrast, bacteriostatic activity was considered when the reduction in CFU/ML was less than 99.9% (<3 $\log_{10}$) or when bacterial growth remained stable **(Pankey & Ashcraft, 2009)**

## IV. Evaluation of the gastroprotective effect

### IV.1 Animal material

All experiments were conducted on adult male Mus musculus. mice, weighing between 25 and 41 g, obtained from the animal facility of the faculty of SNV/STU at the university of 8 may 1945 Guelma. The animals were housed in polypropylene cages under controlled conditions (22 ± 2°C, 12 h light/dark cycle) with ad libitum access to food and water, expect for a 24-hour fasting period before the experiment. All procedures involving animals were carried in accordance with institutional and national guidelines for the care and use of laboratory animals (National Research Council, 2011).

### IV.2 Experimental design

Following the adaptation phase, the mice were sorted by weight, and divided into seven groups of five, all treatments were administered orally via gavage. The groups received the following treatments:

**Table 2**: Group Allocation Based on the Type of Treatment Administered

| Groups | Experimental Condition | Treatment |
|---|---|---|
| **1** | Healthy Control | Received distilled water only. |
| **2** | 96 % Ethanol control | Received ethanol (96%, 0.1 mL/mouse) 1 hour later |
| **3** | Omeprazole (10mg/kg)+96 % Ethanol | Received omeprazole (20 mg/kg), then ethanol 96%,(0.1mL/mouse) 1 hour later |
| **4** | Aqueous extract of *S.alexandrina* (100mg/ml) | Received *Senna alexandrina* extract (100 mg/ml) followed by distilled water 1 hour later, with no ethanol. |
| **5** | Aqueous extract of *S.alexandrina* (200mg/ml)+ | Received *Senna alexandrina* extract (200 mg/ml) followed by distilled water 1 hour later, with no ethanol |
| **6** | Aqueous extract of *S.alexandrina* (100mg/ml)+ Ethanol 96 % | Received *Senna alexandrina* extract (100 mg/ml) followed by ethanol 96%, (0.1mL/mouse)1 hour later. |
| **7** | Aqueous extract of *S.alexandrina* (200mg/ml)+ Ethanol 96 % | Received *Senna alexandrina* extract (200 mg/ml) followed by ethanol 96%, (0.1 mL/mouse)1 hour later. |

Animals were killed at the end of the treatment period by cervical dislocation

### IV.3. Determination of Gastric Mucosal Injury Index

Animals were euthanized at the end of the treatment period by cervical dislocation, following euthanasia, the stomachs were carefully extracted from the animal's abdominal regions. The stomachs were then meticulously washed with sterile distilled water to ensure the removal of blood residue and other extraneous substances that could potentially compromise the quality of the tissue and the accuracy of the lesion assessment.

After this step, the stomachs were meticulously divided along their length in a longitudinal direction. the extent of gastric mucosal injury was assessed using a scoring system based the size and severity of visible lesions.

The gastric ulcer index (GUI) was determined according to the Guth standard **(Guth, Aures, and Paulsen, 1979; El-Maraghy, Rizk, and Shahin, 2015)**

Spot erosions were assigned a score of 1 point. Erosions with a length of <1 mm were scored as 2 points, those between 1-2mm received 3 points. Those between 2-mm were assigned 4 points, and erosions >3 mm were given 5 points. The score was doubled if the erosion width was >1 mm.

The protection percentage was calculated using the following formula **(Palomino-Pacheco *et al.*, 2022).**

$$\%\,\boldsymbol{Protection} = [(\boldsymbol{GUI\_vehicle} - \boldsymbol{GUI\_extract}) / \boldsymbol{GUI\_vehicle}] \times \mathbf{100}$$

Where:

GUI vehicle: represents the gastric ulcer index in the negative control group.

GUI extract: represents the gastric ulcer index in the group treated with the plant extract.

**IV.4. Histological Study**

In order to achieve optimal penetration of the fixative solution and ensure uniform fixation of the tissue, the stomachs were meticulously divided along their length in a longitudinal direction. The tissue samples were placed directly into a 10% neutral formalin solution and left at ambient temperature for a 24-hour period. The samples occurred using the standard histological method **(Arab *et al.,* 2015).** Following fixation, the tissues were dehydrated through a series of ethanol concentrations (70%, 80%, and 100%) to ensure complete removal of any residual water. They were cleared by soaking them in xylene in order to remove any residual ethanol and to prepare the tissues for paraffin embedding. The tissues were then infiltrated with molten paraffin to embed the samples. The paraffin was then cooled and solidified, resulting in the formation of a block that immobilized the tissue.The paraffin-fixed tissues were cut into 5 µm thick sections using a microtome to produce thin samples slices for analysis. The sections were subjected to hematoxylin and eosin (H&E) staining. Sections were subjected to gradual desiccation at ambient temperature or in an oven set to between 37 and 40°C for a period of 15 to 20 minutes. This was undertaken to ensure effective fixing of the staining and complete drying of the samples prior to mounting. Following desiccation, the tissue sections were mounted on glass slides with a mounting medium to ensure the preservation of the tissue and facilitate the process.

The stained slides were subsequently examined under a light microscope to conduct a histopathological analysis, with the objective of ascertaining the tissue architecture and identifying any pathological alterations. To confirm the inflammation and perform a quantitative analysis of inflammatory cells, histological images were captured using a digital microscope and subsequently analyzed with ImageJ software. This approach proved instrumental in the quantitative analysis of inflammatory cells and the subsequent comparison of data across diverse experimental groups.

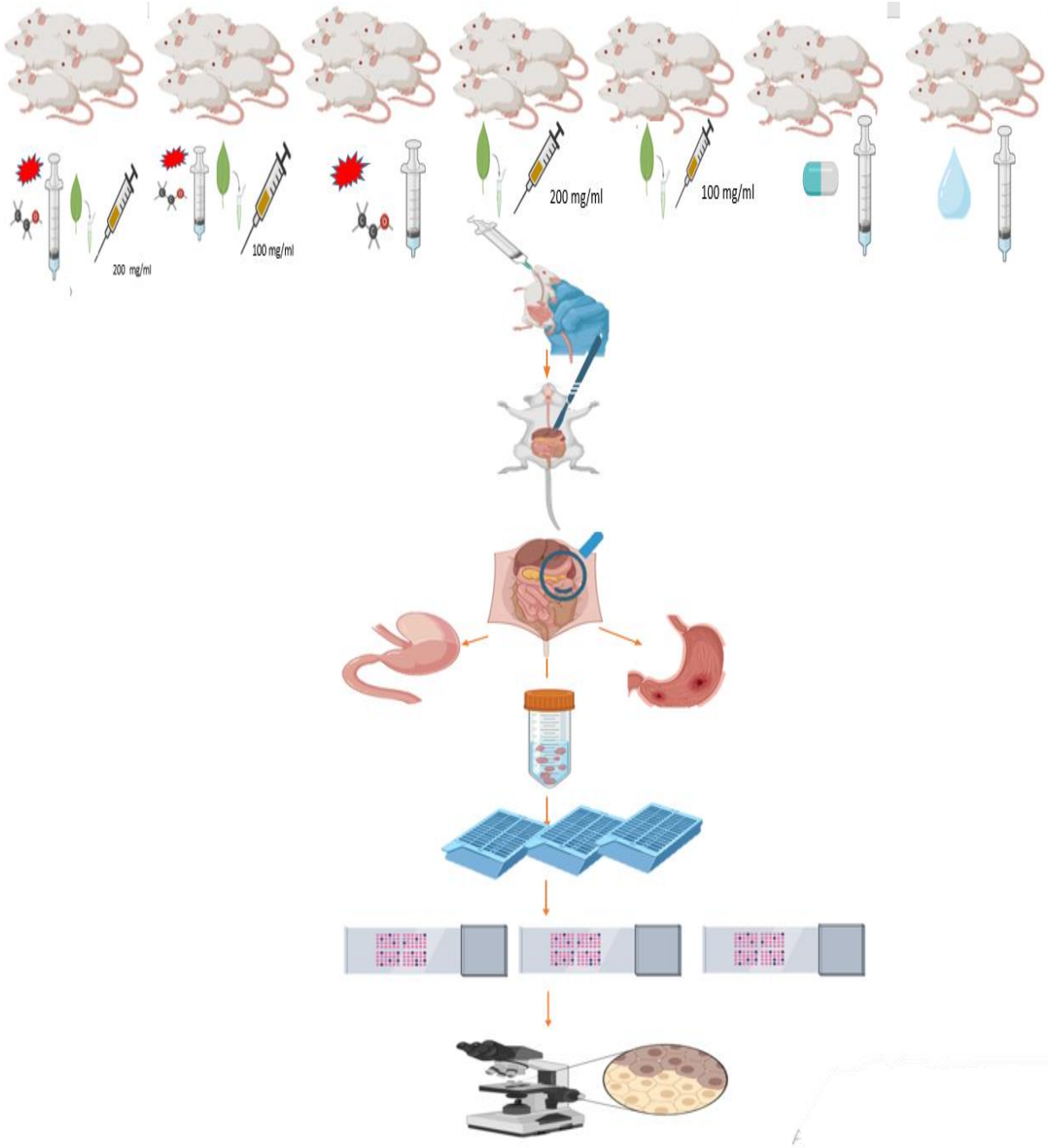


**Figure 6:** Graphical abstract of the experimental protocol for the in vivo animal study

**V. Molecular docking study**

Docking of molecules is a recent bioinformatics technique that predicts the likely experimental orientation as well as the binding affinity required to establish a stable complex structure between a ligand and a target. **(Kumar and S. Kumar, 2019).**

The study of molecular docking of the organic molecules that have been extracted from the plant was performed to complement and confirm the results in the experimental section for the in vitro and in vivo activities using Auto Dock Vina program. The proteins used to simulate these activities, named CPx-ATPase and polymerase, has been obtained from the RCSB database with the code [2YJ5 ] (Hejdanek & Pachl, 2020)and [6SYI] respectively (Voellmecke *et al*., 2012)

The active site of these proteins used is delimited by a box of dimensions: a volume of 40 × 40 × 40 Å3 and a center x, y, z: -2, 1 and -23 for. 2YJ5 and a volume of 40 × 40 × 40 Å3 and a center x, y, z: -15,15 and 30 for [6SYI] .To ensure an effective simulation, the most abundant ligands . sennosides A/B and isocitrate were initially prepared by optimizing it using the CHem3D 16 0 R5 to achieve a stable geometry with minimum energy. Next, the receptors (proteins) for the two activities were prepared using Discovery Studio. This involved removing water molecules and heteroatoms, and adding polar hydrogen (Rizwana *et al.,* 2021). Afterwards, molecular docking simulations were conducted using the AutoDock Vina program (Trott & Olson, 2010).

# *Conclusion*

## Conclusion

In light of the growing interest in plant derived therapeutics with multifunctional properties, this study provides valuable insights into the potential of Senna alexandrina as a natural source of bioactive compounds. The investigation aimed to evaluate the phytochemical profile as well as the antibacterial and gastroprotective potential of the aqueous leaf extract.

The main findings can be summarized as follows:

- Phytochemical analysis using LC-MS QTOF confirmed the presence of major active constituents, notably sennosides and glycosylated flavonoids. FTIR spectroscopy further revealed functional groups characteristic of phenolic and flavonoid structures, such as O-H, C=O, and C-O bonds.
- Quantitative analysis revealed a high content of polyphenols ($28.42 \pm 0.4$ mg GAE/g), flavonoids ($9.05 \pm 0.84$ mg QE/g), and tannins ($0.43 \pm 0.026$ mg CE/g).
- Antibacterial testing demonstrated a dose-dependent inhibitory effect against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, with a bacteriostatic mode of action confirmed by the time-kill assay
- Gastroprotective effect were validated in vivo, where showing significant protection against ethanol-induced gastric lesions in mice, with improved mucosal integrity and reduced inflammation, particularly at the 200 mg/kg
- Molecular docking studies supported the experimental findings, by predicting strong interactions between major phytoconstituents and both bacterial and gastric protein targets, highlighting possible mechanisms behind the extract's dual protective effects.

The accumulated evidence highlights the therapeutic promise of Senna alexandrina as a natural source of bioactive compounds with both antibacterial and gastroprotective effects. These findings open promising avenues for future research, including:

- Isolation and structural characterization of the most active compounds to determine their individual contributions
- Evaluation of the toxicity and safety profile of the extract to assess its clinical potential.
- Investigation of synergistic effects among its phytochemicals using purified compounds and standardized combinations
- Exploration of formulation strategies (e.g., nanoparticles, capsules) to enhance bioavailability and therapeutic efficacy.

In conclusion, Senna alexandrina represents a valuable candidate in the search for plant-based therapeutic agents, particularly for addressing bacterial infections and gastric mucosal disorders.

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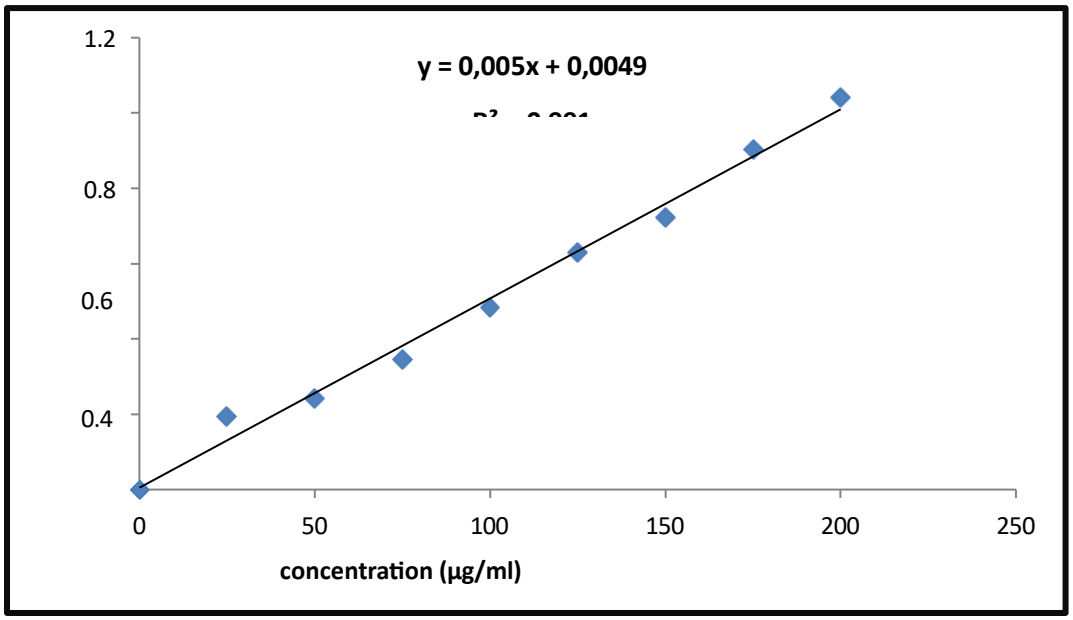
# Annexes

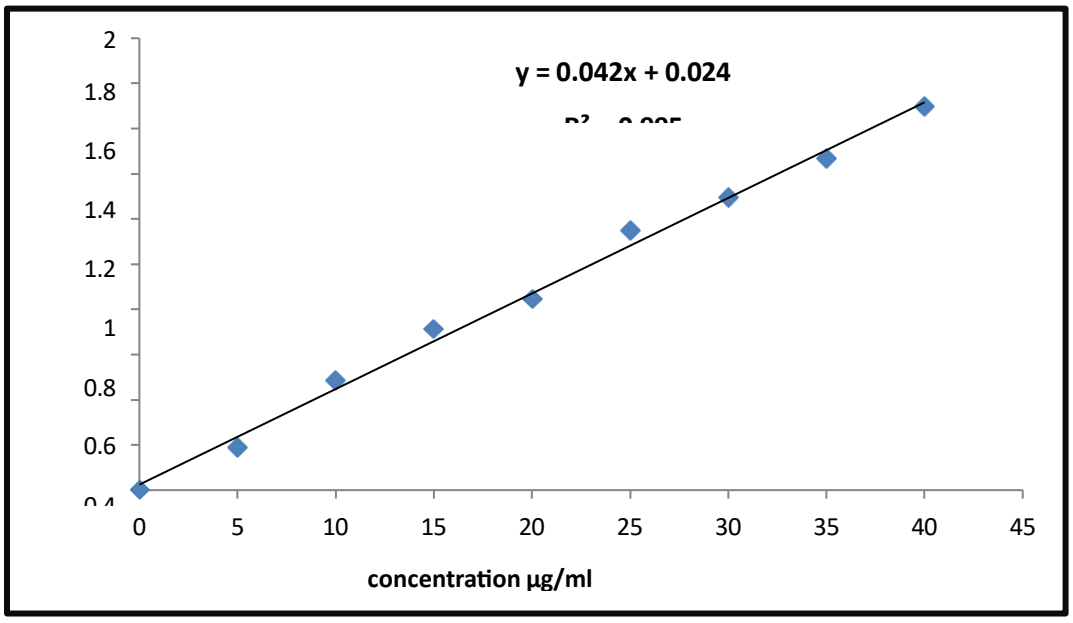
**Annexes**

**Calibration Curve for polyphenols Content Determination**



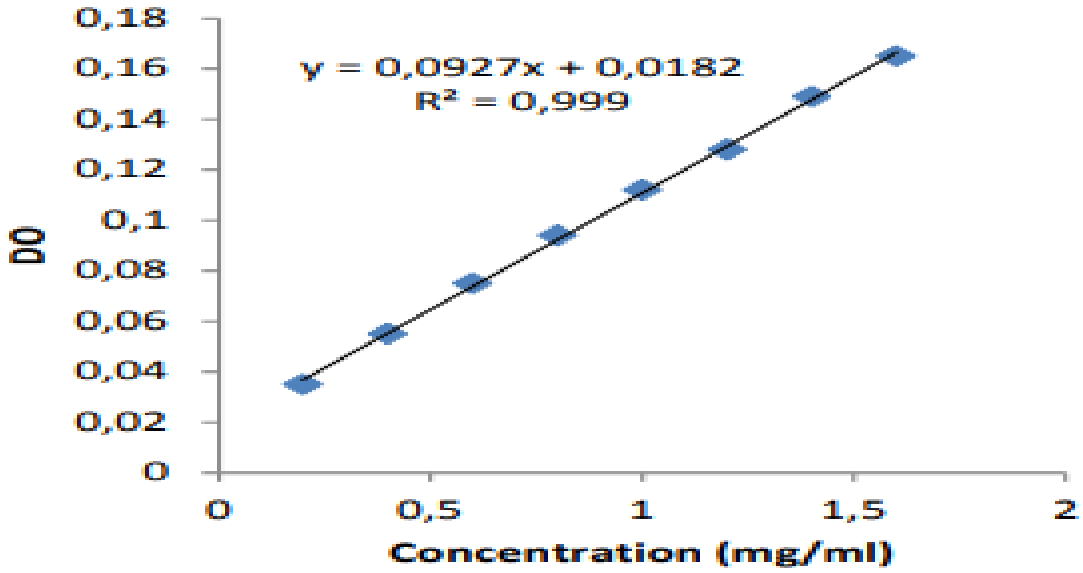

**Annex 1:** Calibration curve for the determination of polyphenols using curve of gallic acid

**Calibration Curve for Flavonoid Content Determination**




**Annex 2:** Calibration Curve for Flavonoid Content Using Quercetin Standard

**Calibration Curve for Condensed tannins content Determination**




**Annex 3:** Calibration Curve for Condensed Tannins Content Using catechin Standard