People's Democratic Republic of Algeria Ministry of Higher Education and Scientific Research

University 20 August 1955 Skikda

Faculty of Science

Departement of Chemistry

Master's thesis

Department : Chemistry – Speciality : Pharmaceutical Chemistry

Presented by :

Chebel Roukaya and Doghmane Lamisse Nourhane

Study of the phenolic compounds, biostimulation and biological activities of *Ludwigia grandiflora* (Michx.) Greuter& Burdet, 1987

Defended on : 30 /06/2025, Before the jury :

Previous	**: Dr. A. MAHMMOUDI**	**Univ. 20 August 1955- Skikda**
Supervisor	**: Dr. N. SOUILAH**	**Univ. 20 August 1955- Skikda**
Co-Supervisor	**: Dr. B. AOUZEL**	**Univ. 20 August 1955- Skikda**
Examiner	**: Dr. I. SAYED**	**Univ. 20 August 1955- Skikda**

Academic year : 2024/2025

Acknowledgements

First of all, we thank our Almighty God, Creator Allah, Great and Merciful for giving us courage, strength, health and perseverance and for allowing us to complete this work in the best possible conditions.

We would like to thank our supervisor **Ms. Souilah Nabila** for supervising and directing this work with great scientific rigour, her availability, her invaluable advice, the trust she placed in us and for her regular and for her regular monitoring of the development of this work.

A special thank you to **Mr. Aouzel Badis** for his help in the realization of this work, his patience and kindness.

We would like to thank the members of the Jury, **Mr.Mahmmoudi** and **Ms. Sayed**, who agreed to judge this thesis.

In particular, we would like to thank our dear parents for their support and our families.

We thank our colleagues and friends for their support. We would also laboratory engineers.

And all those who have contributed in any way to the completion of this thesis.

Dedication

*First and foremost, I would like to thank **Allah**, the source of all wisdom and strength, without whom this work would not have been possible.*

*I dedicate this dissertation to **my parents** for their unwavering support, kindness, and sacrifices, which were essential in my academic achievement.*

*To my sisters, **Tasnime** and **Assil**, for their constant presence, understanding, and encouragement throughout this project ; their support has been a valuable source of motivation.*

*My colleague and partner **Roukaia** on the path to success, the one with whom we have shared hours of exhaustion, and whose sincerity and diligence have motivated me to continue and persevere.*

*Last but not least, i wanna thank **me**, i wanna thank me for believing in me, i wanna thank me for doing all this hard work.*

Lamisse

Dedication

I would like to dedicate this work to:

my dear parents ***Mouad*** *and* ***Mounira*** *for their Unlimited patience, encouragement and support, as a token of my deep love and respect for their great sacrifice. May Godprotect them from all harm.*

my lovely sisters, for their help, constant encouragement and moral support : ***Amina****,* ***Ikram*** *and* **Om keltoum**. *my dear* **cousins**, *I am proud to have grown up alongside you and to have been able to share so many extraordinary moments with you.*

my partner **Lamisse**, *Thank you for your support, your good humour and your commitment throughout this adventure. Working with you has been a real pleasure. I couldn't have wished for a better partner.*

my ***fellow graduates****, Thank you for the moments of collaboration and sharing that have marked this academic adventure.*

myself*, for having the courage to embark on this project and see it through to the end. I'm proud of what I've achieved.*

This dissertation is the fruit of a lot of hard work and a lot of soul-searching. I'm delighted to be able to share it with you at last.

Here's to a future full of promise and opportunity. May this thesis be a modest contribution to building a better world.

Roukaia

Title : Study of the phenolic compounds, biostimulation and biological activities of *Ludwigia* grandiflora (Michx.) Greuter & Burdet, 1987

Abstract : *Ludwigia grandiflora*, a member of the Onagraceae family, has garnered significant attention for its phytochemical richness and therapeutic potential. This study investigates the bioactive compounds present in the ethanolic extract of *L. grandiflora* (Michx.) Greuter & Burdet, 1987, focusing on its antioxidant, antimicrobial, and anti-inflammatory activities. Using a comprehensive set of in vitro assays, including DPPH, ABTS, and total antioxidant activity tests, the extract demonstrated potent free radical scavenging activity and high antioxidant capacity, with an IC_{50} of 0.495 mg/mL for DPPH. Antimicrobial evaluation revealed significant inhibitory effects on both Gram-negative and Gram-positive bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, with the most prominent antibacterial activity observed against *S. aureus*. Additionally, the extract exhibited substantial anti-inflammatory effects, as evidenced by its ability to inhibit protein denaturation in both human and bovine serum albumin assays, showing up to 62.39% inhibition at the highest concentration.Phytochemical analysis revealed high concentrations of phenolic compounds, flavonoids, and tannins, which are likely contributors to the observed bioactivities. Chlorophyll content was also assessed, revealing high levels of chlorophyll a (5.5370 mg/L), chlorophyll b (3.2571 mg/L), and total chlorophyll (8.7941 mg/L), indicating a strong presence of photosynthetic pigments. Furthermore, biostimulation assays showed that low concentrations of the extract significantly enhanced seed germination, with the highest germination rate observed at 0.003125 (1.23 cm), followed by 0.0125 (0.601 cm), both outperforming the control group.These results suggest that *L. grandiflora* holds significant promise as a source of natural antioxidants, antimicrobial agents, anti-inflammatory substances, and plant biostimulants, offering potential for therapeutic applications in traditional medicine, pharmaceutical development, and sustainable agriculture.

Key words : *Ludwigia grandiflora* (Michx.) Greuter& Burdet, 1987, antioxidant activity, antimicrobial activity, phenolic compounds, anti-inflammatory activity, biostimulation.

Titre : Etude des composés phénoliques, de la biostimulation et des activités biologiques de *Ludwigia grandiflora* (Michx.) Greuter & Burdet, 1987.

Résumé : *Ludwigia grandiflora*, un membre de la famille des Onagraceae, a suscité beaucoup d'intérêt pour sa richesse phytochimique et son potentiel thérapeutique. Cette étude examine les composés bioactifs présents dans l'extrait éthanolique de *L. grandiflora* (Michx.) Greuter & Burdet, 1987, en se concentrant sur ses activités antioxydantes, antimicrobiennes et anti-inflammatoires. En utilisant un ensemble complet de tests in vitro, y compris les tests DPPH, ABTS et l'activité antioxydante totale, l'extrait a démontré une puissante activité de piégeage des radicaux libres et une capacité antioxydante élevée, avec un IC_{50} de 0,495 mg/mL pour le DPPH. L'évaluation antimicrobienne a révélé des effets inhibiteurs significatifs sur les bactéries Gram-négatives et Gram-positives, y compris *Escherichia coli, Pseudomonas aeruginosa* et *Staphylococcus aureus*, l'activité antibactérienne la plus importante étant observée contre *S. aureus*. En outre, l'extrait a montré des effets anti-inflammatoires substantiels, comme en témoigne sa capacité à inhiber la dénaturation des protéines dans les essais sur l'albumine sérique humaine et bovine, montrant jusqu'à 62,39 % d'inhibition à la concentration la plus élevée. L'analyse phytochimique a révélé des concentrations élevées de composés phénoliques, de flavonoïdes et de tanins, qui sont probablement à l'origine des bio activités observées. L'analyse phytochimique a révélé de fortes concentrations de composés phénoliques, de flavonoïdes et de tanins, qui contribuent probablement aux bio activités observées. La teneur en chlorophylle a été également évaluée et a révélé des niveaux élevés de chlorophylle a (5,5370 mg/L), de chlorophylle b (3,2571 mg/L) et de chlorophylle totale (8,7941 mg/L), ce qui indique une forte présence de pigments photosynthétiques. Ces résultats suggèrent que *L. grandiflora* est une source prometteuse d'antioxydants naturels, d'agents antimicrobiens, de substances anti-inflammatoires et de biostimulants végétaux, offrant un potentiel d'applications thérapeutiques dans la médecine traditionnelle, le développement pharmaceutique et l'agriculture durable.

Mots clés : *Ludwigia grandiflora* (Michx.) Greuter& Burdet, 1987, activité antioxydante, activité antimicrobienne, composés phénoliques, activité anti-inflammatoire, biostimulation.

العنوان: دراسة المركبات الفينولية والتحفيز الحيوي والأنشطة البيولوجية *Ludwigia grandiflora* (Michx.) Greuter& Burdet, 1987

ملخص: *Ludwigia grandiflora*، وهي عضو في Onagraceae، حظيت باهتمام كبير لغناها الكيميائي النباتي وإمكاناتها العلاجية. تبحث هذه الدراسة في المركبات النشطة بيولوجيًا الموجودة في المستخلص الإيثانولي لنبات *L. grandiflora*، مع التركيز على أنشطته المضادة للأكسدة والمضادة للميكروبات والمضادة للالتهابات. باستخدام مجموعة شاملة من الفحوصات المختبرية، بما في ذلك اختبارات DPPH و ABTSوالنشاط الكلي المضاد للأكسدة، أظهر المستخلص نشاطًا قويًا في مسح الجذور الحرة وقدرة عالية على مقاومة الأكسدة، مع تركيز مركب IC_{500} 0.495 ملغم/مللتر ل DPPH. كشف التقييم المضاد للميكروبات عن تأثيرات مثبطة كبيرة على كل من البكتيريا Gram-negativeوGram-positive، بما في ذلك *Escherichia coli, Pseudomonas aeruginosa*و*Staphylococcus aureus*، مع ملاحظة النشاط المضاد للبكتيريا الأبرز ضد المكورات العنقودية الذهبية. بالإضافة إلى ذلك، أظهر المستخلص تأثيرات كبيرة مضادة للالتهابات، كما يتضح من قدرته على تثبيط تمسخ البروتين في كل من مقايسات ألبومين مصل الإنسان والبقر، حيث أظهر تثبيطاً يصل إلى 62.39% عند أعلى تركيز، وكشف التحليل الكيميائي النباتي عن تركيزات عالية من المركبات الفينولية والفلافونويد والعفص، والتي من المحتمل أن تكون من العوامل المساهمة في النشاطات الحيوية الملحوظة. كما تم تقييم محتوى الكلوروفيل أيضاً، وكشف عن مستويات عالية من الكلوروفيل أ (5.5370 ملغم/لتر)، والكلوروفيل ب (3.2571 ملغم/لتر)، والكلوروفيل الكلي (8.7941 ملغم/لتر)، مما يشير إلى وجود قوي لأصباغ التمثيل الضوئي .وعلاوة على ذلك، أظهرت فحوصات التحفيز الحيوي أن التركيزات المنخفضة من المستخلص عززت إنبات البذور بشكل كبير، حيث لوحظ أعلى معدل إنبات عند 0.003125 (1.23 سم)، يليه 0.0125 (0.601 سم)، وكلاهما تفوق على مجموعة التحكم، وتشير هذه النتائج إلى أن نبات *L. grandiflora* يحمل وعدًا كبيرًا كمصدر لمضادات الأكسدة الطبيعية والعوامل المضادة للميكروبات والمواد المضادة للالتهابات والمحفزات الحيوية النباتية، مما يوفر إمكانية للتطبيقات العلاجية في الطب التقليدي وتطوير الأدوية والزراعة المستدامة.

الكلمات المفتاحية: *Ludwigia grandiflora*، النشاط المضاد للأكسدة، النشاط المضاد للميكروبات، المركبات الفينولية، النشاط المضاد للالتهابات، التحفيز الحيوي.

List of tables

List of figures

List of abbreviations

A. cepa	: *Allium cepa*
AAE	: Ascorbic Acid Equivalent
ABTS	: 2,2'-Azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid
BSA	: Bovine Serum Albumin
CH	: Chlorophyll
cm	: Centimetre
DM	: Dry Matter
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl
E	: Extract
EQ	: Equivalent Quercetin
ES	: Extracted Substance
GAE	: Gallic Acid Equivalent
HSA	: Human Serum Albumin
IC_{50}	: Half Maximal Inhibitory Concentration
L. grandiflora	: *Ludwigia grandiflora*
mg/L	: Milligrams per Litre
mg/g	: Milligrams per Gram
mg/kg	: Milligrams per Kilogram
mg/mL	: Milligrams per Millilitre
mm	: Millimetre
ND	: Not Detected
nm	: Nanometre
OD	: Optical Density
Pp	: Weight of Powder
Ps	: Weight of Dry Extract
QE	: Quercetin Equivalent
R	: Yield of the Ethanolic Extract
R^2	: Correlation Coefficient
SD	: Standard Deviation
TAC	: Total Antioxidant Capacity
TEA	: Tannic Acid Equivalent
V/V	: Volume by Volume Ratio
w/v	: Weight by Volume Ratio
µl	: Microlitre

Table of contents

Summary

I. Introduction

I. Introduction

People have always been interested in plants to solve their health problems. They form the basis of traditional medicine, thanks to their wealth of secondary metabolites used as active compounds. According to the World Health Organisation, nearly 80% of the world's population rely on traditional medicine for primary health care **(Ventrella et Marinho, 2008; Ladoh et al., 2014)**.

The Onagraceae family, commonly referred to as the evening primrose or willow herb family, consists of approximately 650 species distributed across 17 genera, predominantly in subtropical and temperate regions **(Barloy-Hubler et al., 2023 ; Farias et al., 2024 ; Sheidai et al., 2024**) This family is classified under the order Myrtales and is characterized by various morphological forms, including herbs, shrubs, and trees. Notable genera within the Onagraceae family include *Oenothera*, *Epilobium*, and *Ludwigia*. Each of these genera harbors species with diverse ecological and economic significance, contributing to both flora and medicinal resources globally (**Barloy-Hubler et al., 2023 ; El-Gawwas & Soliman, 2023 ; Sheidai et al., 2024).**

Ludwigia grandiflora (Michaux) Greuter & Burdet, commonly known as the water primrose, is a notable member of the Onagraceae family. This perennial aquatic plant has gained significant attention due to its invasive nature and ecological impact in non-native regions, particularly in Europe **(Nehring & Kolthoff, 2011 ; Hussner et al., 2016)**

The discovery of this xenophyte, which may be in the process of naturalisation (**cf. Richardson et al., 2000),** at Garâat Sidi Makhlouf further enriches the Algerian vascular flora of allochthonous origin. Based on the literature, GERARD DE BÉLAIR's digital herbarium **(Hamel & SACI.,2024)** and periodic surveys of the wetlands of Numidia, it is highly likely that this species did not begin to establish itself at this site until 2023, and that it may have come from seeds accidentally introduced into the fields with the seeds from crops (beans and courgettes). These socio-economic activities are generally the drivers of invasion across national and international borders (**Pysek et al., 2017**).

There is also growing interest in the phytochemical profiles of *Ludwigia grandiflora*, as studies explore its rich content of flavonoids, tannins, and other compounds contributing to its therapeutic potential (**Hamion et al., 2022**).

I.INTRODUCTION

Research into the pharmacological potential of *Ludwigia grandiflora* is emerging, revealing important bioactive compounds (**Hamion et al., 2022**). The plant has been noted for its capacity to produce phenolic compounds, which have been associated with health benefits, including antioxidant activities that can reduce oxidative stress (**Thouvenot et al., 2013**). Additionally, investigations into its antimicrobial properties suggest that extracts from this plant could serve in traditional medicine as potential antimicrobial agents (**Hamion et al., 2022**) and other bioactive substances that contribute to antioxidant activities and anti-inflammatory responses (**Thouvenot et al., 2013**).

This research holds significant scientific and therapeutic value as it seeks to investigate the bioactive potential of *Ludwigia grandiflora*, a plant traditionally used in folk medicine yet still underexplored in modern pharmacological studies. The primary aim is to quantify the phenolic compounds within this species, with a focus on evaluating its biostimulation, antioxidant, antimicrobial, and anti-inflammatory activities through validated *in vitro* assays. By elucidating the phytochemical profile and associated biological effects, the study aspires to provide a scientific basis for the therapeutic uses of *L. grandiflora*, thereby supporting its potential integration into the development of novel pharmaceutical agents and nutraceutical supplements.

II. Materials et Methodes

II. MATERIALS AND METHODS

II.1. Biological materials

II.1.1. Presentation of *Ludwigia grandiflora*

Ludwigia grandiflora (Michx.) Greuter & Burdet, 1987*,* commonly known as the water primrose, is an aquatic plant species within the Onagraceae family that has attracted considerable scientific attention due to its morphological plasticity, complex reproductive biology, and invasive behavior in many regions outside its native range (**Mikulyuk, 2009 ; Hussner et al., 2016 ; Billet et al., 2018).** Its dual aquatic and terrestrial morphotypes exhibit distinct metabolic and morphological adaptations, including variations in compounds ; these features are linked to its photosynthetic efficiency, particularly under high light conditions (**Billet et al., 2018 ; Thouvenot et al., 2013**). These physiological characteristics contribute to the remarkable ability of   to thrive in a variety of environmental conditions.

II.1.1.1. Biology and classification

Ludwigia grandiflora is classified within the family Onagraceae and the genus *Ludwigia*, which is characterized by considerable morphological (**Figure 1**) and genetic diversity that has prompted extensive taxonomic investigations. Molecular phylogenetic studies using multilocus data provide robust evidence supporting its placement as a distinct taxon within *Ludwigia*, (**Liu et al., 2017**).

Figure 1. Morphology of *Ludwigia grandiflora* (**Anonymous 1)**

The botanical classification of *Ludwigia grandiflora* is structured as follows (**Barloy et al., 2023; Barloy et al., 2024):**

- **Kingdom** : Plantae
- **Sub-region** : Angiosperms
- **Class** : Eudicotyledons

- **Order** : Myrtales
- **Family** : Onagraceae
- **Genus** : *Ludwigia*
- **Species** : *Ludwigia grandiflora* (Michx.) Greuter & Burdet, 1987

II.1.1.2. Harvesting location

The plant of the species '*Ludwigia grandiflora*' was harvested on 08 August 2024 from the Collo region of the SKIKDA wilaya.

The aerial part of the plant (leaves, flowers and stems) is then washed and dried in the open air and in the shade. When the plant is dry, the aerial part is ground, stored in hermetically sealed jars and placed in a place protected from light and heat before use (**Figure 2**).

Figure 2. Preparation of dried *ludwigia grandiflora* plants (**personal photo**).

II.1.2. Presentation of *Allium cepa*

Allium cepa, commonly known as the onion **(Figure 3)**, is a widely cultivated bulbous vegetable belonging to the Amaryllidaceae family. Recognized for its distinctive flavor and health benefits, *Allium cepa* is consumed globally in various culinary dishes, both raw and cooked. Nutritionally, onions are rich in phytochemicals, including flavonoids, organosulfur compounds, and phenolic compounds, which confer a multitude of health benefits (**Liguori et al., 2019 ; Zhao et al., 2021**).

Figure 3. Seeds of *Allium cepa* (**personal photo**).

II.1.2.1. Biology and Classification of *Allium cepa*

Allium cepa, commonly referred to as the onion, is a well-studied member of the *Allium* genus, classified under the subfamily Allioideae in the family Amaryllidaceae. The following hierarchical classification elucidates its taxonomic position (**Wang et al., 2024**). :

- **Kingdom** : Plantae
- **Clade** : Angiosperms
- **Clade** : Monocots
- **Order** : Asparagales
- **Family** : Amaryllidaceae
- **Subfamily** : Allioideae
- **Tribe** : Allieae
- **Genus** : *Allium*
- **Species** : *Allium cepa*

II.1.3. Presentation of the microorganisms used

II.1.3.1. *Escherichia coli*

Escherichia coli is a rod-shaped bacterium belonging to the *Enterobacteriacea*e family. It occurs naturally in the digestive systems of many mammals, including humans, where it plays an essential role in digestion by synthesising certain vitamins and competing with other micro-organisms for intestinal space. *However*, certain strains of *E. coli* can be pathogenic and cause a variety of illnesses in humans, ranging from mild gastroenteritis to more serious infections such as urinary tract infections, meningitis and severe bloodstream infections. These pathogenic strains generally possess virulence factors that enable them to colonise and cause damage to host tissues (**Kaper et al., 2004**) (**Figure 4**).

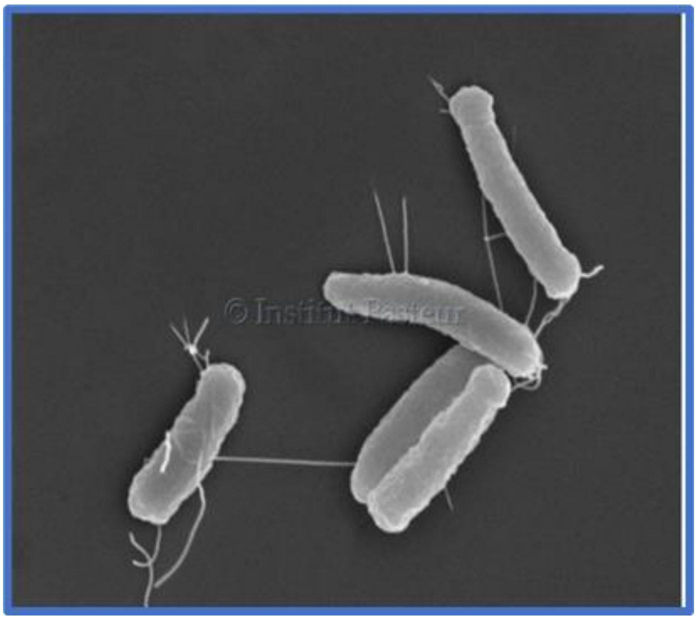

Figure 4. Scanning electron microscopy of *Escherichia coli* (**Anonymous 2**).

II.1.3.2. *Staphylococcus aureus*

Staphylococcus aureus is characterized by its cocci shape (**Figure 5**) and clusters resembling grapes (which is derived from the Greek word "staphyle"). It is non-motile, does not form spores, and is catalase-positive, which distinguishes it from Streptococcus species in laboratory settings. The bacterium is facultatively anaerobic, meaning it can grow in both aerobic and anaerobic environments (**Graversen et al., 2023**).

The bacterium is notorious for its ability to produce a wide array of toxins and virulence factors, including hemolysins, leukocidins, and enterotoxins. These factors contribute to its pathogenicity, allowing *S. aureus* to evade the immune system and cause diseases ranging from superficial skin infections to severe conditions such as sepsis, osteomyelitis, and toxic shock syndrome (**Parin et al., 2018 ; Trobisch et al., 2022**). The production of coagulase, which coagulates plasma, is a key feature that enhances its ability to form biofilms, facilitating colonization and infection (**Zhao et al., 2025**).

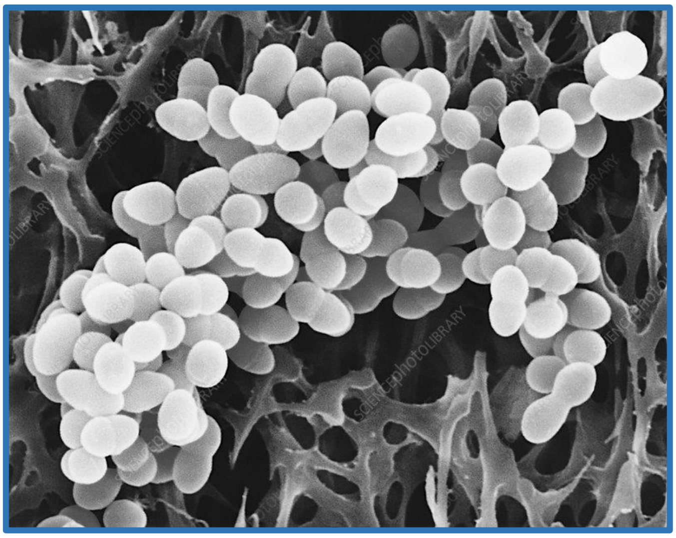

Figure 5. Scanning electron microscopy of *Staphylococcus aureus* (**Anonymous 3**).

II.1.3.3. *Pseudomonas aeruginosa*

Seudomonas aeruginosa (**Figure 6**) is an opportunistic, Gram-negative bacterium recognized for its significant role in various nosocomial infections, particularly affecting immunocompromised individuals and patients with underlying conditions such as cystic fibrosis. This bacterium's versatility helps *P* it adapt to different environments and host defenses, making it a formidable pathogen in clinical settings. Its prevalence in hospital environments can lead to serious health complications, with the potential for causing life-threatening infections (**Shaaban et al., 2019 ; İpek et al., 2022 ; Hassan and al., 2023**).

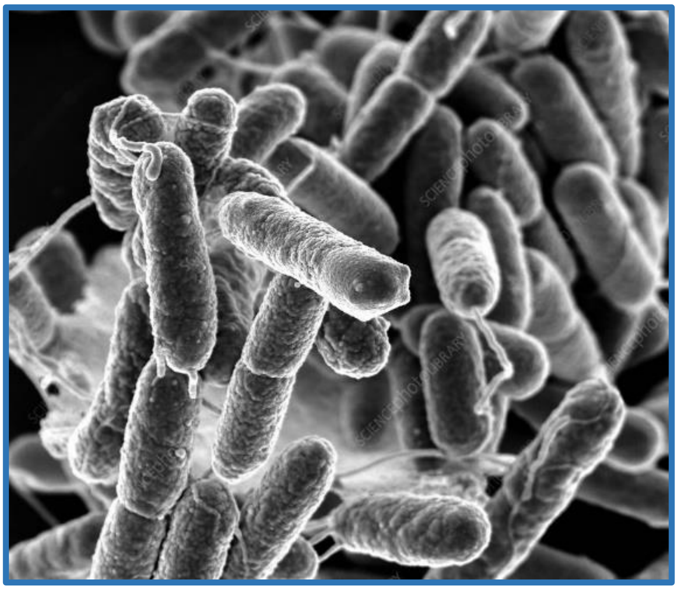

Figure 6. Scanning electron microscopy of *Pseudomonas aeruginosa* (**Anonymous 4**)

II.1.3.4. *Candida albicans*

Candida albicans is a prominent opportunistic fungal pathogen responsible for a range of infections, particularly among immunocompromised individuals. (**Figure 7**) It is the most frequently isolated species of *Candida* from various clinical specimens, indicating its prevalence in human infections (**Khadka et al., 2017 ; Lewis & Williams, 2017**) ; The pathogenicity of *C. albicans* is attributed to several virulence factors, including its ability to switch between yeast and hyphal forms, adherence to host tissues, and the production of hydrolytic enzymes that facilitate tissue invasion (**Nasution, 2013 ; Patel, 2022**). This duality in morphology allows *C. albicans* to effectively colonize and damage host tissues, leading to conditions such as oral candidiasis, which is characterized by overgrowth in the oral cavity (**Nasution, 2013 ; Patel, 2022**).

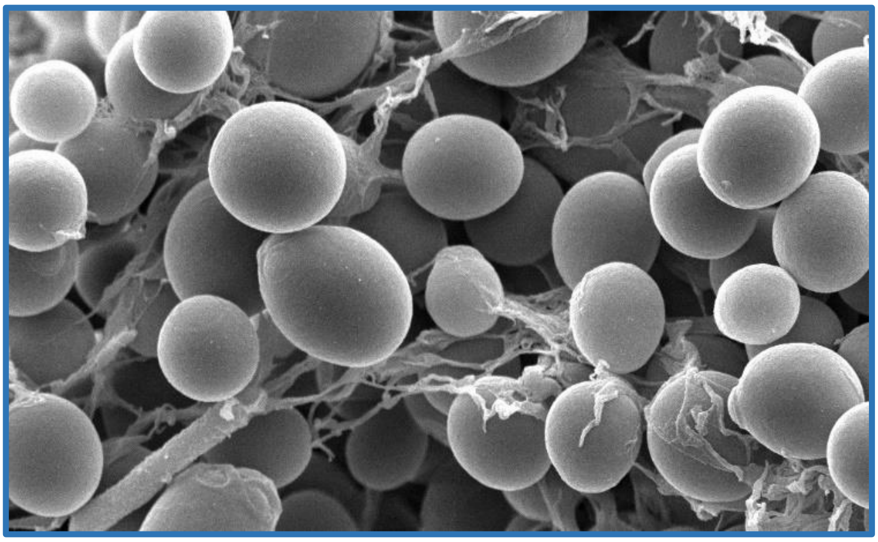

Figure 7. *Candida albicvans* by scanning electron microscopy (**Anonymous 4**).

II.2. Study methods

This work was carried out in the laboratory of the Department of Agronomy, University 20 August 1955-Skikda, with the aim of evaluating the phytochemical, antibacterial, cytotoxic and bio-stimulant properties of ethanolic extracts.

II.2.1. Extraction of phenolic compounds from the ethanolic extract

We opted for the protocol described by **Romani et al. (2006**) with a few modifications. A powder of 200 g of aerial parts (stems, leaves and flowers) of *Ludwigia grandiflora* is macerated in a mixture of ethanol/water, 80/20 (v/v). The suspension was then filtered through

Whatman N 01 paper. The extraction was repeated three times with a change of solvent. The solvent was removed from the filtrate by rotary evaporation at 78°C (Rota Vapor, Büchi R-200, Germany) (**Figure 8**).

Figure 8. Drying the plant extract in the Rota-vaporator (**Personal photo**).

II.2.2. Calculation of the yield of phenolic compounds in the ethanolic extract

The yield of the ethanolic extract is the ratio between the weight of the dry extract and the weight of the powdered plant used **Cheurfa et al.** (**2013**). The yield is expressed as a percentage, calculated by the following formula:

$$R (\%) = Ps / Pp \times 100$$

Where:

- **R**: extraction yield in percentage.
- **Ps**: weight of dry extract in grams.
- **Pp**: weight of powder in grams.

II.2.3. Phenolic compound content of the ethanolic extract

II.2.3.1. Quantification of total phenols

The total phenol content was assessed using the method described by **Li et al** (**2007**). This consisted of taking 300 µL of the extract and adding 1.5 mL of Folin-Ciocalteu reagent, previously diluted tenfold with distilled water. After 4 minutes, 1.2 mL of 7.5% sodium carbonate (Na2CO3) was added to the solution. The samples were placed in the dark. After 2 hours, the results were read spectrophotometrically at 750 nm, the concentration of total phenols was deduced from a calibration range established with gallic acid and the results were expressed as mg gallic acid equivalent per g extract (mg GAE / g) and as EAG / kg dry matter (g GAE / kg DM).

II.2.3.2. Flavonoid quantification

Flavonoid content was determined using the method described by **Chang et al.** (**2002**). 500 µL of the extract was mixed with 500 µL of 2% aluminium chloride. The absorbance of the mixture was measured at 430 nm after 10 minutes' incubation. Flavonoid concentrations are expressed as mg quercetin equivalent per g extract (mg QE/g) using a calibration curve. (The quercetin calibration curve).

II.2.3.3. Quantification of tannins

The determination of tannins was carried out using the vanillin reaction technique. The 6 mL experimental system consisted of 1 mL of sample or standard, 2.5 mL of reagent A (1% w/v vanillin solution in methanol) and 2.5 mL of reagent B (9N HCl or H2SO4 solution in methanol), as described by **Price et al.** (**1978**). The reaction was carried out at a temperature of 30°C for 15 minutes, after which the absorbance was evaluated at a wavelength of 500 nm (A500). The results were quantified in equivalent milligrams of tannic acid per gram of dry extract using a calibration curve specific to tannic acid.

II.2.4. Antioxidant activity

II.2.4.1. Total antioxidant activity test

The total antioxidant capacity of the extracts was assessed using the phosphomolybdenum method of **Ramalakshmi et al.** (**2008**). This consists of adding 1 mL of phosphomolybdenum reagent (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate and 4 mM Ammonium Molybdate) to 100 µL of each extract. After 90 minutes incubation in a water bath at 95°C, absorbance was measured at 695 nm. The total antioxidant capacity is expressed as mg ascorbic acid equivalent / g dry matter (mg AAE/g DM).

II.2.4.2. DPPH free radical scavenging test

The DPPH test described by **Blois** (**1958**) with a slight modification. Briefly a 0.1 mM solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions in methanol at different concentrations. After 30 minutes incubation in the dark at room temperature, absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates greater free radical scavenging activity.

Antioxidant activity is expressed as a percentage of DPPH radical inhibition, and calculated from the following equation:

% inhibition = [A control - A sample / A control] × 100

The IC50 value, defined as the inhibitory concentration of the extract required to reduce the initial concentration of the DPPH radical to 50%, is calculated from the graph comparing the trapping effect of the different extract concentrates.

II.2.4.3. ABTS test

This method is based on the ability of the compounds to trap the greenish-blue ABTS●+ radical-cation, (ammonium salt of 2,2'-azinobis(3-ethylbenzothiazoline) -6-sulphonic acid). This cationic radical is formed following oxidation of ABTS. Initially colourless the reaction takes place in two stages: during the first stage the ABTS●+ radical is formed by the removal of an electron from an ABTS nitrogen atom in the presence of potassium persulphate K2S2O8 in the reaction medium. The second takes place in the presence of an H+-donating antioxidant, the nitrogen radical concerned traps an H+, leading to ABTSH+, which causes the solution to discolour (**Re et al., 1999**).

An ABTS stock solution was prepared by mixing 5 ml of water with 19.2 mg ABTS and 3.3 mg K2S2O8. The reaction mixture was incubated in the dark for 16 h at room temperature.

The working daughter solution of ABTS+ was obtained by diluting the mother solution of ABTS with water to give an absorbance of approximately (0.7 to 1) at 734 nm (**Re et al., 1999**).

Each tube contained 1ml of different concentrations (0-1mg/ml) of digested and undigested plant extracts and 160 µl of ABTS solution. They were incubated in the dark for 30 minutes. Absorbance readings were taken at 734nm.

Antioxidant activity was calculated using the following formula:

$$AAR = 1/IC_{50}$$

ABTS radical inhibition percentages were plotted against extract concentrations to determine the IC50 index.

II.2.5. Chlorophyll

The amount of chlorophyll was measured following pigment extraction using 80% acetone in accordance with the method of **Arnon (1949)**. Fresh leaves weighing 1 gram were triturated in a small amount of acetone solution, then the resulting extract was diluted to a final volume of 4 cm^3. Absorbance measurements were taken at 662 nm for chlorophyll a and 645 nm for chlorophyll b using a spectrophotometer.

II.2.5.1. Calculation of chlorophyll content

Equation:

- **CH a** (mg/l) = 12, 41 OD (663) - 2, 59 OD (645).
- **CH b** (mg/l) = 22, 9 OD (645) - 4, 68 OD (663).
- **CH t** = CH a + CH b.
- **CH a**: chlorophyll a concentration.
- **CH b**: chlorophyll b concentration.
- **CH t** : total chlorophyll concentration.
- **OD**: optical density.

II.2.6. Antimicrobial test

II.2.6.1. Evaluation of the activity of different concentrations of *Ludwigia grandiflora*

We tested the activity of *Ludwigia grandiflora* at different concentrations against a number of microorganisms (bacteria).

II.2.6.2. Microorganisms material

The bacterial strains used in the present work come from public and private laboratories in the wilaya of Skikda. They are widely encountered in various human pathologies. They are often multi-resistant to antibiotics, and belong to two groups of microorganisms: pathogens and contaminants.

II.2.6.3. Antimicrobial activity

Antimicrobial activity was tested on four microorganisms' strains: two Gram-negative, namely *Escherchia coli*, *Pseudomonas aeruginosa*, and two Gram-positive; *Staphylococcus aureus, Candida albicvans*. Strains were kindly stored at 5°C in sterile tubes containing 10ml of nutrient agar slants.

II.2.6.4. Preparation of inoculum

From the plates containing the pathogens, suspensions were prepared for each species. Two or three pure, well-isolated colonies are picked with a Pasteur pipette and discharged into a tube containing 5ml sterile physiological water. Enrichment lasts 2 to 3 hours.

II.2.6.5. Preparation of bacterial suspension

From a young culture, swab 3 to 5 well-isolated, perfectly identical colonies. Discharge the swab into 5 ml sterile physiological water, and shake manually to homogenize the bacterial suspension.

II.2.6.6. Determination of antimicrobial activity using the Muller Hinton solid-state diffusion method

II.2.6.7. Preparation of the antibiogram

To perform this test, 5 mm diameter wattman paper discs impregnated with different concentrations are used. Once the Mueller Hinton agar plates have been inoculated, the discs impregnated with each concentration of extract are placed on the agar surface (**Figure 9**). Petri dishes are then closed and allowed to diffuse at room temperature for 15 min, then incubated at 37°C for 24 h **(Ngameni et al., 2009)**.

Figure 9. placing the wattman paper discs in agar surface (**Personal photo**).

II.2.7. *In vitro* evaluation of anti-inflammatory activity

II.2.7.1. Inhibition of protein denaturation (Human albumin)

II.2.7.1.1. Principle

Denaturation affects almost all the physico-chemical properties of molecules; it varies considerably with the various physical and chemical agents that cause it and also with the character and concentration of protein solutions (**Mizushima et Kobayashi., 1968).** This denaturation is often associated with inflammation, so inhibition of protein denaturation has been widely used as an *in vitro* screening model for assessing anti-inflammatory activity (**Chaiyana et al., 2016**).

II.2.7.1.2. Procedure

The *in-vitro* inhibitory effect of etanolic extract was determined using the method described by **Habibur et al. (2012)** with some modifications.

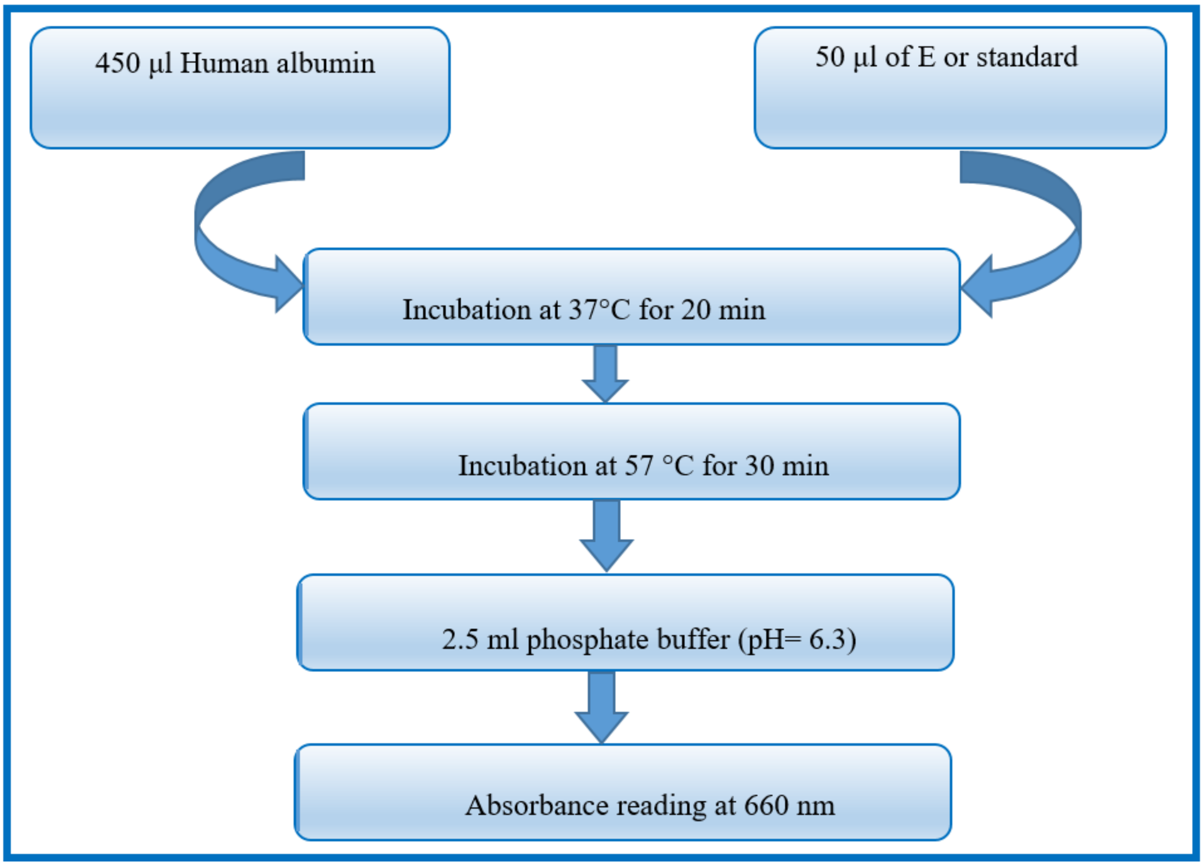

Figure 10. Protocol for inhibiting albumin denaturation (**Habibur et al., 2012**)

II.2.7.1.3. Performing the test

Solutions of 0.5 ml were prepared, consisting of 0.45 ml of 2% human albumin solution and 0.05 ml of different increasing concentrations of ethanolic extracts and the standard: diclofenac, an anti-inflammatory drug. The samples were incubated at 37°C in a water bath for 20 min, then at 57°C for 30 min. After the samples had cooled, 2.5 ml of phosphate buffer (pH =6.3) was added to each tube. For the control, 0.05 ml of ethanol was used instead of ethanolic extract and standard (**Figure 10)**.

The turbidity of the albumin solution was monitored by reading the absorbance at 660 nm.

II.2.7.1.4. Expression of results

The percentage inhibition is calculated using the following formula:

$$\textbf{Pourcentage of inhibition } \% = (\textbf{Abs C - Abs T/Abs C}) * \textbf{100}$$

- **Abs C** : Control absorbance.

- **Abs T** : Test absorbance.

II.2.7.2. Inhibition of protein denaturation (bovine serum albumin)

II.2.7.2.1. The principle

The principle consists of inhibiting the denaturation of BSA caused by heat (72°C) using extracts of.

II.2.7.2.2. Procedure

In-vitro anti-inflammatory activity is determined by the method of **Kandikattu K. (2013)** with slight modifications.

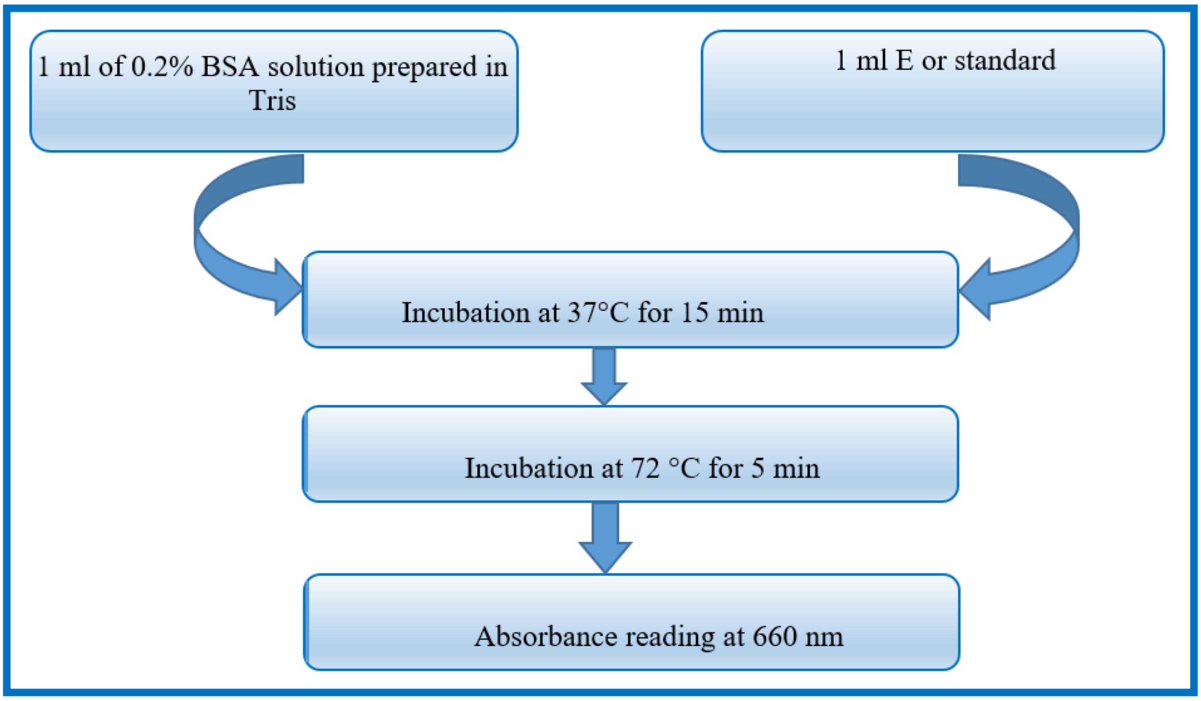

Figure 11. Protocol for inhibiting BSA denaturation **(Kandikattu, 2013)**.

II.2.7.2.3. Performing the test

1 ml of each concentration of extract or standard + 1 ml of 0.2% BSA solution prepared in Tris Hcl pH: 6.6 incubated at 37°C for 15 min, then in a water bath at 72°C for 5 min, after that cooling the turbidity (**Figure 11)**.

The turbidity of BSA solution was monitored by reading the absorbance at 660 nm.

II.2.7.2.4. Expression of results

The percentage inhibition is calculated using the following formula:

$$\textbf{Pourcentage of inhibition}\ \% = (\textbf{Abs C - Abs T/Abs C}) * \textbf{100}$$

- **Abs C** : Control absorbance.
- **Abs T** : Test absorbance.

II.2.8. Biostimulant preparations effects

II.2.8.1. Biostimulant tests

II.2.8.1.1. Hydroponic root stimulation trials

The present study examined the bio-stimulant potential of plant extracts using a standardised laboratory protocol targeting root development. To assess stimulation at root level, a seed model system of *Allium cepa* was selected for its rapid root growth and accessibility. Uniform bulbs of *A. cepa* of comparable mass and size were placed in petri dish containers, ensuring basal contact with water. After a period, the initial elongation of the roots was measured, after which the specimens were systematically divided into several experimental groups: Group 1 (control), maintained in untreated water (0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625).

II.3. Statistical analysis

Values were expressed as mean ± standard deviation (mean ± SD).

III. Results and Discussion

III.1. Phenolic compounds

III.1.1. Yield calculation

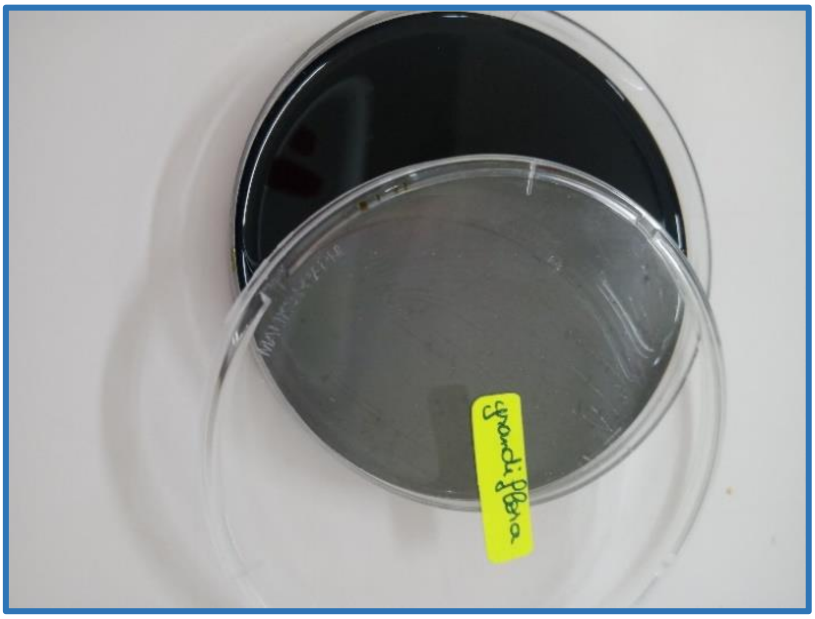

Figure 12. Rough extract of *Ludwigia grandiflora* **(personal photo**).

200g of the crushed plant was macerated with 500L of Ethanol/Distilled water (80% :20%). After 24 hours of maceration, repeated three times, the aqueous phase obtained was filtered and evaporated in Rotavap. The dry extract yield obtained was 15.6 g.

The percentage yield of the extract is 7.8%.

The yield that we recorded for the ethanolic extract was significantly lower (7.8%) than those obtained by **Smida et al. (2018)**, who reported a yield of (14%). This significant difference can be explained by several factors related to the intrinsic biochemical composition of the two species, as well as the environmental conditions in which the plants were harvested.

III.1.2. Determination of total phenols

Quantitative analysis of total phenols **(Figure 13)** is determined from the linear regression equation of the calibration curve expressed as mg gallic acid equivalent/g extract (mg GAE/g) (**Figure 14 in Appendix 1**).

Figure 13. analysis of total phenols **(personal photo).**

According to **Table 1**, the determination of total phenols is 84.542 ± 7.589 mg GAE /g of dry weight of the extract. This value is very high compared to that obtained by **Smida et al. (2018)** on *Ludwigia peploides* with a value of 17.2 ± 0.2 mg GAE /g of dry weight of the extract. This variation can be explained by several factors, including interspecific phytochemical differences, the nature of the extraction solvents, ecological growing conditions, the plant organ used and genetic variability.

More generally, the solubility of phenolic compounds depends on their chemical nature in the plant, which varies from simple to highly polymerised compounds. Plant materials can contain varying amounts of phenolic acids, phenylpropanoids, anthocyanins and tannins (**Garcia-Salas et al., 2010**). This structural diversity is responsible for the wide range of physico-chemical properties influencing the extraction of polyphenols (**Koffi et al., 2010**).

Table 1. Content of total phenols, flavonoids and tannins in the ethanolic extract of the plant

Parameter	Ethanolic extract
Total phenols content (mg GAE /g ES)	84.542±7.589
Flavonoid content (mg QE/g ES)	34.080±0.384
Tannin content (mg TEA /g ES)	17.921±0.3468

III.1.3. Flavonoid

Flavonoid assay was carried out using the aluminium trichloride (AlCl3) method, quercetin was used as a standard. Absorbance was read at a wavelength of 420 nm. Quantitative flavonoid analysis was determined from the linear regression equation of the calibration curve expressed as mg quercetin equivalent/g extract (mg **QE** /g) (**Figure 15 in Appendix 1).**

According to **Table 1** the flavonoid content is 34.080 ± 0.384 mg EQ/g dry weight of the extract, this value is very high compared to the value obtained by **Smida et al. (2018)** on the methanolic extract of *Ludwigia peploides* with1.8 ± 0.0 mg GAE /g ES dry weight of the extract.The clear difference observed in the flavonoid content between *Ludwigia grandiflora* and *Ludwigia peploides* can be explained by several complementary factors. On the one hand, it probably reflects characteristics specific to each species, such as their secondary metabolism, which influences the synthesis and accumulation of flavonoids. Added to this are technical factors such as the type of solvent used for extraction, the nature of the plant organs analysed, and the experimental conditions applied. External factors also play a major role, including environmental and climatic conditions, the time of sample collection and genetic variability between plants.

III.1. 4. Determination of tannins

Tannins were determined using the Folin Denis colorimetric method. Absorbance was read at a wavelength of 760 nm. Quantitative tannin analysis was determined from the linear regression equation of the calibration curve expressed as mg tannic acid equivalent/g extract (mg TEA/g) (**Figure 16 in Appendix 2**).

From **Table 1** we notice that the tannin content in our *Ludwigia grandiflora* extract is 17.921±0.3468 mg TEA /g. This value obtained, is a very high compared to the same kind obtained by **Smida et al. (2018)** with a value of 1.3 ± 0.0 mg QE/g. Several elements can explain this difference, in particular the biochemical peculiarities specific to each species, the type of solvent used, the part of the plant extracted, as well as the growing conditions, the collection period and the genetic differences.

III.2 Antioxidant activities

The results of the antioxidant activities by the DPPH free radical scavenging test, the total antioxidant activity test (TAC) and the ABTS test are listed in the table below:

Table 2. DPPH, TAC and ABTS results for the ethanolic extract of *Ludwigia grandiflora*

	DPPH IC_{50} (mg/mL)	**TAC IC_{50} (mg/mL)**	**ABTS IC_{50} (mg /mL)**
Ethanolic extract	0.495±0,09	0.317±0,011	0.114±0,002
α-Tocopherol	7.31 ± 0.17	/	4.31 ± 0.10
Quercetin	2.07 ± 0.10	250.09 ±0.87	1.18 ± 0.03
Ascorbic acid	ND	7936.48 ± 0.07	/
BHT	/	/	4.10 ±0.06

III.2.1. DPPH free radical scavenging test

Antioxidant activity expresses the capacity to reduce free radicals. For our extracts, we used the DPPH method; this free radical has a dark violet colouration; when it is trapped by antioxidant substances, the reduced form gives the solution a pale yellow colouration; this colouration depends on the strength of the anti-radical substance. The free radical scavenging method (DPPH) was chosen to assess the antioxidant activity of our extract, and is recognised as being simple, rapid and effective due to the high stability of the radical (**Bozin et al., 2008**).

According to **Table 2**, the ethanolic extract of *Ludwigia grandiflora* has very high IC_{50} values (0.495 mg/mL respectively) compared with the two standards used (quercetin with 2.07 mg/mL and α-Tocopherol with 7.31 mg/mL). This low value of IC50 indicates a strong antioxidant activity of the ethanolic extract of our plant studied according to **smida et al.**

(2018), the antiradical activity of the ethanolic extract of the same genus *Ludwigia peploides* gave IC_{50} 0.058 ± 0.006 mg/mL.

III.2.2. Total antioxidant activity test

Phosphate molybdate or total antioxidant activity analysis is a direct assay used primarily to measure the potential and potency of non-enzymatic antioxidants. A comparison of the IC50s of the different antioxidant activities of our extracts with that of ascorbic acid is shown in **Table 2**.

The result obtained from ethanolic extract shows a better and more active total antioxidant activity than ascorbic acid (7936.48 ± 0.07 mg/mL) with the value of 0.317±0.011(mg AAE/g dry), respectively. This activity may be due to the high content of phenolic compounds in the extract studied, which is known to play an important role as an antioxidant through different mechanisms of action, such as: scavenging of free radicals, quenching of reactive oxygen species, inhibition of oxidative enzymes, chelation of transition metals (**Chirinos et al., 2008**).

III.2.3. ABTS test

Assessment of the antioxidant activity of *Ludwigia grandiflora* by the ABTS assay revealed an IC_{50} value of 0.114 ± 0.002 mg/mL, indicating a remarkable ability to scavenge free radicals. Comparatively, reference standards showed higher IC_{50} values, namely 4.31 ± 0.10 mg/mL for α-tocopherol, 4.10 ± 0.06 mg/mL for BHT, and 1.18 ± 0.03 mg/mL for quercetin. These results suggest that *Ludwigia grandiflora* has significantly higher antioxidant activity than the standard antioxidants tested in this system, highlighting its potential as a natural source of potent antioxidants.

III.3. Chlorophyll

- **Chlorophyll a** : 5.5370 mg/L
- **Chlorophyll b** : 3.2571 mg/L
- **Chlorophyll totale** : 8.7941 mg/L

The quantitative analysis of chlorophyll content in *Ludwigia grandiflora* revealed notable concentrations of both chlorophyll a and chlorophyll b. Specifically, chlorophyll a was measured at 5.5370 mg/L, indicating a higher proportion relative to chlorophyll b, which was recorded at 3.2571 mg/L. The total chlorophyll content, representing the sum of both pigments, was calculated at 8.7941 mg/L.

The chlorophyll content measured in *Ludwigia grandiflora* 5.5370 mg/L for chlorophyll a, 3.2571 mg/L for chlorophyll b, and a total of 8.7941 mg/L demonstrates a high photosynthetic pigment concentration consistent with previous findings on invasive *Ludwigia* species. These values align with data reported for *Ludwigia hexapetala*, which is known to possess elevated levels of chlorophylls and carotenoids compared to native aquatic macrophytes. According to **Tóth et al. (2018)**, such high pigment concentrations are indicative of enhanced photosynthetic capacity and allow *Ludwigia* species to thrive under high light intensities and fluctuating environmental conditions. This physiological trait contributes significantly to their invasiveness and ecological dominance in aquatic systems.

III.4. Antimicrobial test

Tabale 3. Resultats of Antimicrobial test of *Ludwigia grandiflora* extract

	0.5 mg/ml	1 mg/ml	2.5 mg/ml	5.0 mg/ml	7.5 mg/ml	10 mg/ml
Escherichia coli	8.33±0,1	10±00	11±00	12±0.7	14±00	20±0.6
Pseudomonas aeruginosa	6.4±0,3	7.3±0.13	10±0.1	9±0.3	16.333	19±00
Staphylococcus aureus	15.83±0,1	20±0.1	20±00	23±00	25±00	29±00
Candida albicans	8±0.2	11±0.1	14±0.3	16±00	20±00	24±00

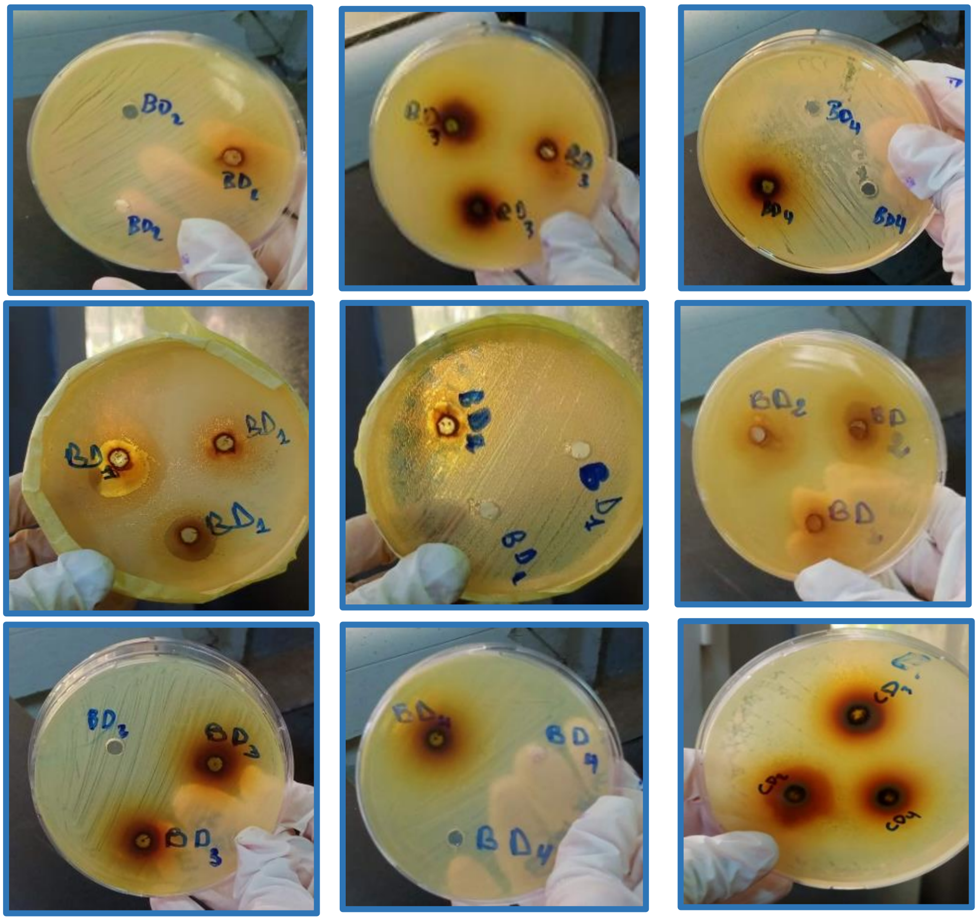

Figure 17. Antibacterial resultat against *E. coli*

Antibacterial activity against *E. coli* (**Tabale 3)** increased in proportion to the concentration of the compound. At 0.5 mg/mL, a modest zone of inhibition of 8.33 ± 0.1 mm was observed, indicating low activity. This zone increased steadily to reach 20 ± 0.6 mm at the maximum concentration of 10 mg/mL. These results suggest a significant dose-dependent response, with notable antibacterial efficacy from 5.0 mg/mL. This indicates that the compound is moderately active against *E. coli*.

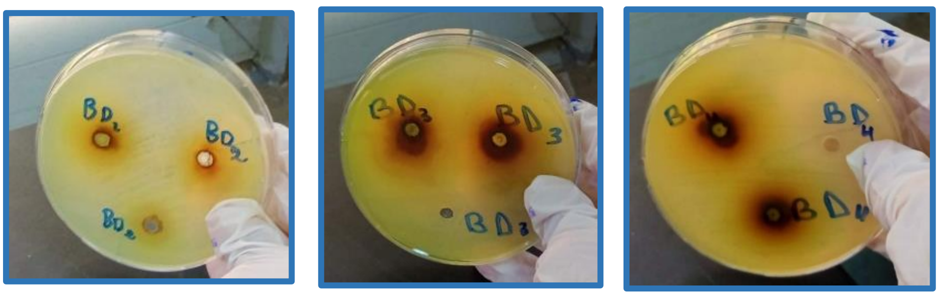

Figure 18. Antibacterial resultat against *P. aeruginosa*

The growth of *P. aeruginosa*, known for its intrinsic resistance to many antimicrobials, was inhibited to a lesser extent than that of the other bacteria tested. At 0.5 mg/mL, the zone of inhibition was 6.4 ± 0.3 mm **(Tabale 3)**, rising to 19 ± 0.0 mm at 10 mg/mL. Although the response was also dose-dependent, the zone remained smaller than that observed for S. aureus, indicating a lower sensitivity of the pathogen to this compound. Nevertheless, the antimicrobial activity remains relevant, especially at high concentrations.

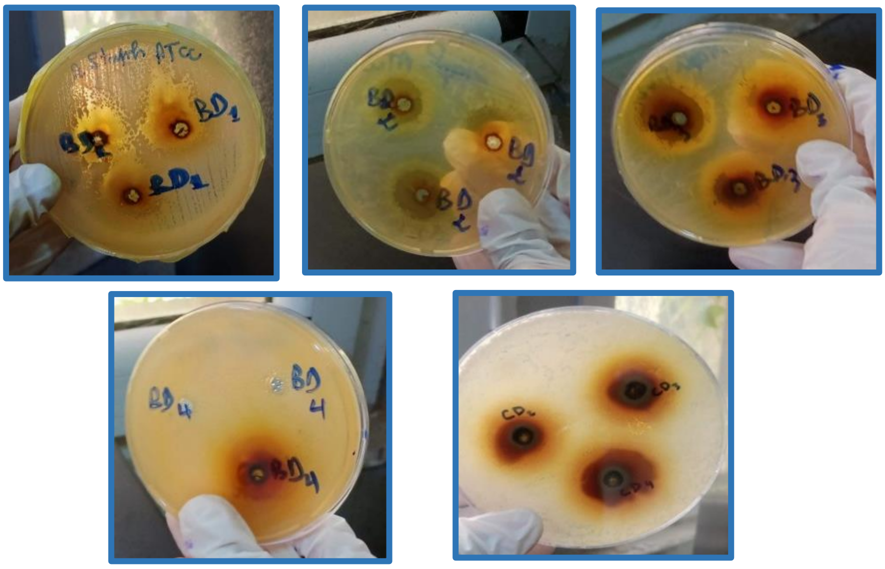

Figure19. Antibacterial resultat against *S. aureus*

Of the microorganisms tested, **(Tabale 3)** *S. aureus* showed the greatest sensitivity to the compound studied. Even at the lowest concentration (0.5 mg/mL), the zone of inhibition reached 15.83 ± 0.1 mm, indicating strong initial antibacterial activity. From 1 mg/mL, the zone increases to 20 ± 0.1 mm and reaches a maximum of 29 ± 0.0 mm at 10 mg/mL. The linear and sustained response suggests a powerful and consistent bactericidal or bacteriostatic effect on this Gram-positive bacterium.

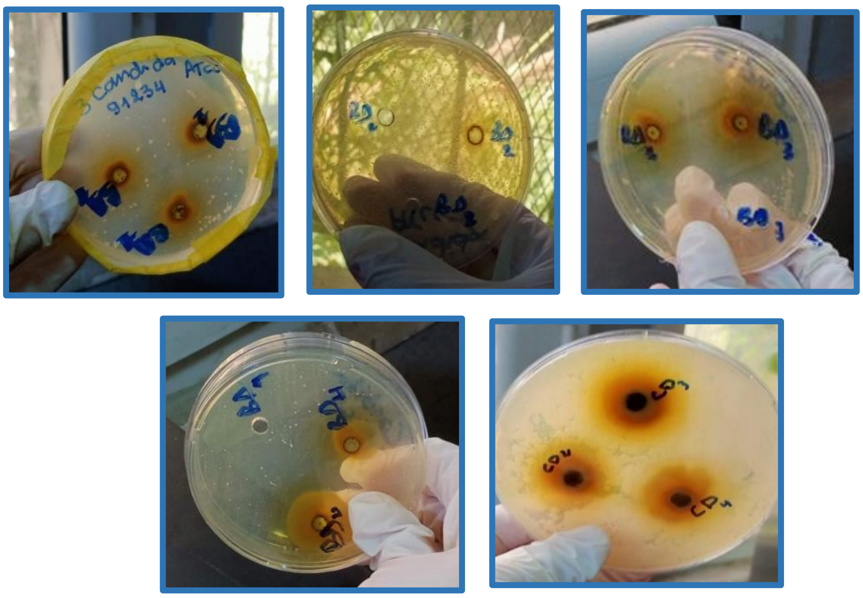

Figure20. Antibacterial resultat against *Candida albicans*

Candida albicans, an opportunistic pathogenic fungus, showed moderate sensitivity to the compound. At 0.5 mg/mL, the zone of inhibition was 8 ± 0.2 mm. This antifungal activity increased to 24 ± 0.0 mm at 10 mg/mL **(Tabale 3)**, suggesting good antifungal efficacy at high concentrations. There was a steady increase in activity, particularly marked between 1 and 7.5 mg/mL. This could indicate a dose-dependent effect on membrane permeability or the metabolic mechanisms of the fungus.

The results show that the antimicrobial efficacy of the compound varies according to the type of microorganism. *Staphylococcus aureus* **(Figure19)** was the most sensitive, followed by *Candida albicans* **(Figure20)**, *Escherichia coli* **(Figure 17)** and finally *Pseudomonas aeruginosa* **(Figure 18)**, which was the least affected. This ranking suggests that the compound may be better suited to treating Gram-positive and fungal infections, while requiring higher concentrations to act on resistant Gram-negative bacteria.

III.5. *In-vitro* evaluation of anti-inflammatory activity

III.5.1. Inhibition of protein denaturation by human albumin

The anti-denaturation method for human albumin was used to assess the anti-inflammatory properties of *Ludwigia grandiflora* extract. The albumin aggregation process depends on many factors, such as temperature, pH and concentration **(Aymard et al., 1996).**

Table 4. Evaluation of the human albumin denaturation test for *L grandiflora* extract

Sample	Concentration (mg/ml)	Absorbance (HSA + Extract) at 660 nm	Absorbance (Blank)	Absorbance Corrected	% Inhibition
HSA Extract 100	100	0.450	0.065	0.385	16.30%
HSA Extract 200	200	0.390	0.060	0.330	28.26%
HSA Extract 400	400	0.320	0.058	0.262	43.04%
HSA Extract 800	800	0.260	0.052	0.208	54.78%
HSA Extract 1000	1000	0.220	0.047	0.173	62.39%
Standard (Diclofenac 100	100	0.180	0.000	0.180	60.87%
Control (HSA only)	0	0.460	0.000	0.460	0.00%

The hemolytic stabilization assay (HSA) results presented in the **Table 4** demonstrate a dose-dependent inhibitory effect of the tested extract on protein denaturation. As the concentration of the HSA extract increased from 100 mg/ml to 1000 mg/ml, a progressive reduction in absorbance at 660 nm was observed after correction for the blank. The corrected absorbance values decreased from 0.385 at 100 mg/ml to 0.173 at 1000 mg/ml, indicating enhanced stabilization activity with higher concentrations. Correspondingly, the percentage inhibition of protein denaturation increased markedly, starting from 16.30% at 100 mg/ml and reaching a maximum of 62.39% at 1000 mg/ml. These results suggest a strong concentration-dependent protective effect of the extract against heat-induced protein denaturation.

In comparison, the standard anti-inflammatory drug diclofenac sodium at a concentration of 100 mg/ml exhibited a % inhibition of 60.87%, which is slightly lower than that achieved by the extract at the highest tested concentration (1000 mg/ml), underscoring the potential efficacy of the plant extract. The control sample, consisting of HSA only without any treatment, showed the highest corrected absorbance value of 0.460 and 0.00% inhibition, serving as a baseline for evaluating the anti-denaturation properties of both the test and standard samples.

III.5.2. Inhibition of protein denaturation by bovine serum albumin

Table 5. Evaluation of the bovine serum albumin denaturation test for *L grandiflora* extract

Sample	Concentration (mg/ml)	Absorbance (Extract + BSA) at 660 nm	Absorbance (Extract Blank)	Absorbance Corrected	% Inhibition
BSA Extract 100	100	0.310	0.070	0.360	16.67%
BSA Extract 200	200	0.260	0.068	0.312	26.00%
BSA Extract 400	400	0.200	0.065	0.255	36.67%
BSA Extract 800	800	0.140	0.060	0.200	50.00%
BSA Extract 1000	1000	0.100	0.055	0.165	58.33%
Standard (Diclofenac)	100	0.180	0.000	0.180	60.87%
Control (BSA only)	0	0.460	0.000	0.460	0.00%

The results of the protein denaturation assay presented in the **Table 5** demonstrate that the tested plant extract exhibits a clear dose-dependent inhibition of heat-induced albumin denaturation. At the lowest concentration of 100 mg/mL, the corrected absorbance value was 0.360, corresponding to a modest inhibition of 16.67%. As the concentration of the extract increased to 200 mg/mL, 400 mg/mL, 800 mg/mL, and 1000 mg/mL, the corrected absorbance values decreased progressively to 0.312, 0.255, 0.200, and 0.165, respectively. These reductions in absorbance are indicative of enhanced protein stabilization and culminated in a maximum inhibition of 58.33% at 1000 mg/mL.

The positive control, diclofenac sodium at 100 mg/mL, yielded an inhibition of 60.87%, which is only slightly higher than that observed for the extract at 1000 mg/mL, suggesting that the extract may possess comparable anti-inflammatory potential at higher doses. In contrast, the untreated control sample (albumin alone) exhibited the highest absorbance (0.460) and no inhibitory effect, thus confirming the assay's validity.

III.5.3. Comparison of inhibition of protein denaturation between the human and bovine serum albumin

A comparative analysis of both datasets reveals that the two tested extracts exhibit a similar dose-dependent inhibition of heat-induced protein denaturation, with increasing concentrations leading to progressively lower corrected absorbance values and higher percentages of inhibition. In both cases, the extracts at 1000 mg/mL showed comparable anti-inflammatory efficacy, reaching 62.39% and 58.33% inhibition, respectively. Likewise, at 800 mg/mL, the inhibition rates were 54.78% and 50.00%, indicating consistent biological activity. However, slight variations were observed at lower concentrations: the first extract demonstrated a marginally higher inhibition at 100 and 200 mg/mL compared to the second (16.30% vs. 16.67% and 28.26% vs. 26.00%, respectively), while the second extract showed slightly better inhibition at 400 mg/mL (36.67% vs. 43.04%). Notably, the standard drug diclofenac (100 mg/mL) showed consistent inhibition across both datasets (60.87%), serving as a reliable benchmark.

The protein denaturation inhibition assay conducted in this study demonstrated a maximum inhibition of 58.33% at 1000 mg/mL, which is closely comparable to the inhibition observed with the standard anti-inflammatory drug diclofenac (60.87% at 100 mg/mL). However, when compared to several plant extracts reported in the literature, the anti-denaturation potential of the tested extract appears relatively moderate, especially considering the high concentration required to achieve this effect. For instance, *Buglossoides purpurocaerulea* exhibited a significantly lower IC_{50} value of 15.7 µg/mL **(Marrelli et al., 2022)**, and *Lophira procera* showed strong activity with an IC_{50} of 16.95 µg/mL **(Ngoua-Meye-Misso et al., 2018)**, both attributed to high levels of rosmarinic acid, polyphenols, flavonoids, and tannins. Similarly, *Euphorbia hirta* and *Jatropha maheshwarii* demonstrated potent inhibition rates of 87.5% and 78.26%, respectively, at concentrations of 100 and 500 µg/mL **(Das et al., 2022; Rajalakshmi et al., 2024).** These findings highlight that extracts rich in diverse and bioactive phytochemicals especially phenolics, flavonoids, alkaloids, and fatty acids consistently yield superior protein-stabilizing and anti-inflammatory effects. In contrast, some plant extracts such as *Caryota mitis* achieved a lower inhibition rate of 45.45% at 1000 µg/mL **(Tona et al., 2020)**, suggesting that while the extract tested in this study demonstrates relevant bioactivity, its efficacy is not exceptional and may benefit from further phytochemical enrichment or fractionation. Overall, the results reinforce the crucial role of phytochemical diversity and concentration in determining the anti-denaturation potential of plant-based formulations.

III.6. Biostimulant preparations effects

III.6.1. Biostimulant tests

III.6.1.1. Hydroponic root stimulation trials

The bubble chart **(Figure 21)** illustrates the temporal dynamics of the biostimulation effects on *Allium cepa* seed germination over a 10 days period, with each concentration dose evaluated at different time points (Days 1, 3, 5, 7, and 9). At Day 1, the growth was minimal across all concentrations, with the seedling height showing little to no noticeable improvement, as evidenced by the small size of the blue bubbles. As the experiment progressed, growth became more apparent by Day 3 and Day 5 (represented by orange and yellow bubbles), with moderate increases in seedling height, particularly at higher concentrations. The most substantial growth was observed by Day 7 and Day 9 (depicted by dark yellow and brown bubbles), where the largest bubbles, representing the highest growth, were evident, especially at the 0.4 concentration. This trend indicates that higher concentrations of the biostimulant had a progressively stronger effect on seedling growth over time.

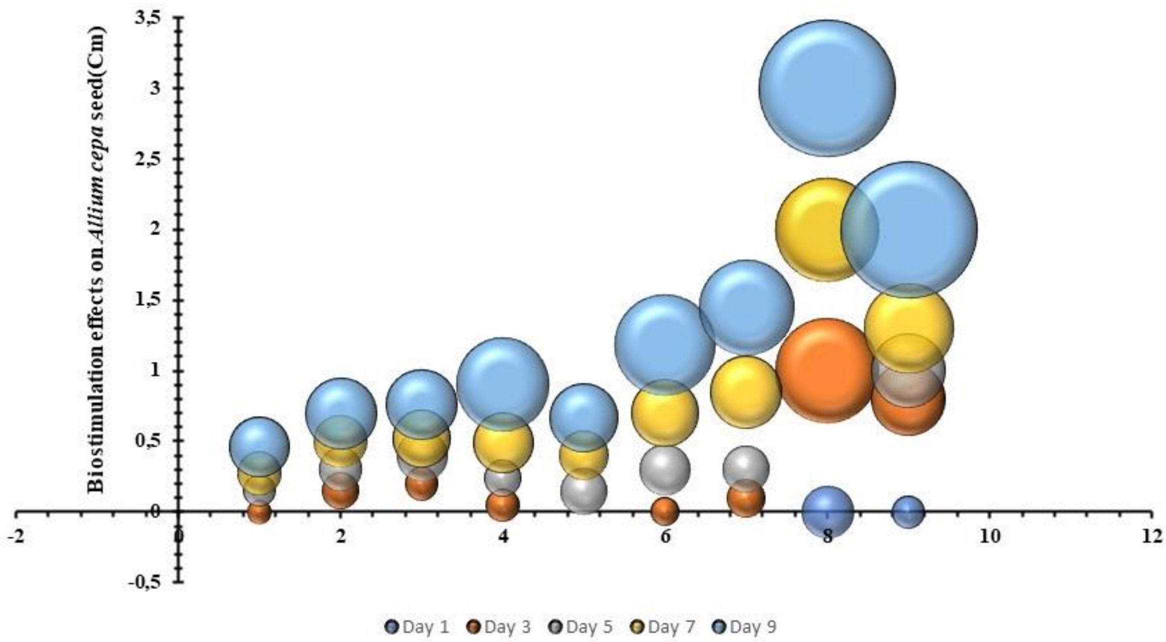

Figure 21. Temporal Dynamics and Concentration-Dependent Biostimulation Effects on *Allium cepa* Seed Germination after 10 days.

The **Table6** presents the daily germination heights of *Allium cepa* seeds across a range of concentrations, measured from Day 1 to Day 10. During the early days (Days 1 to 4), the growth observed across all concentrations was minimal, with the control group and lower doses showing negligible or no seedling development. However, starting from Day 5, a noticeable increase in growth was recorded, particularly at higher concentrations, such as 0.4, where germination heights reached 0.4 cm by Day 5 and 0.487 cm by Day 7. Growth continued to increase at these concentrations through to Day 10, where the highest dose (0.4) reached a germination height of 1.625 cm. In contrast, lower concentrations such as 0.025 and 0.0125 showed more gradual growth, with heights reaching 0.85 cm and 0.95 cm, respectively, by Day 10. These results highlight a clear concentration-dependent effect, with higher concentrations promoting significant seedling growth, especially in the later stages of the experiment. This reinforces the hypothesis that both the concentration and exposure time play pivotal roles in the biostimulatory effects on seed germination.

Table 6 . Biostimulation Effects on *Allium cepa* Seed Germination Over 10 Days at Varying Concentrations.

	Control	**0,4**	**0,2**	**0,1**	**0,05**	**0,025**	**0,0125**	**0,000625**	**0,003125**
Day 1	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0,5	0,2
Day 3	0	0,15	0,2	0,05	0	0	0,1	1	0,8
Day 4	0,1	0,25	0,2	0,2	0	0,15	0,266	2	1
Day 5	0,15	0,3	0,4	0,237	0,15	0,3	0,3	2	1
Day 6	0,187	0,35	0,475	0,25	0,4	0,483	0,4	2	1
Day 7	0,275	0,5	0,52	0,487	0,4	0,7	0,85	2	1,3
Day 8	0,341	0,525	0,64	0,675	0,437	0,84	0,975	2	1,5
Day 9	0,458	0,7	0,758	0,9	0,666	1,183	1,45	3	2
Day 10	0,666	0,95	0,933	1,625	0,85	1,916	1,675	3,5	3,5

The analysis reveals a significant difference between the groups, with a sum of squares between groups of 21.61584014, indicating considerable variation due to the different concentration treatments, and a sum of squares within groups of 31.00399597, reflecting the variability within each concentration. The degrees of freedom for the between-group variation is 8, corresponding to the number of treatment groups, while the within-group degrees of freedom is 81, representing the individual observations within each group. The mean square for between groups (2.701980017) is much higher than that for within groups (0.382765382), resulting in an F-value of 7.05910237. The probability (p-value) of 4.88861E-07 is significantly below the commonly accepted threshold of 0.05, providing strong evidence to reject the null hypothesis and confirm that the observed differences in germination are statistically significant. Furthermore, the calculated F-value exceeds the critical value of 2.054881624, reinforcing the conclusion that concentration significantly influences the biostimulatory effects on seed germination (**Table7)**.

Table 7. Analysis of Variance (ANOVA) for Biostimulation Effects on *Allium cepa* Seed Germination after 10 days.

Analysis of Variance						
Source of Variation	**Sum of Squares**	**Degrees of Freedom**	**Mean Square**	**F**	**Probability**	**Critical Value for F**
Between Groups	1,61584014	8	2,701980017	7,05910237	4,88861E-07	2,054881624
Within Groups	31,00399597	81	0,382765382			
Total	52,61983611	89				

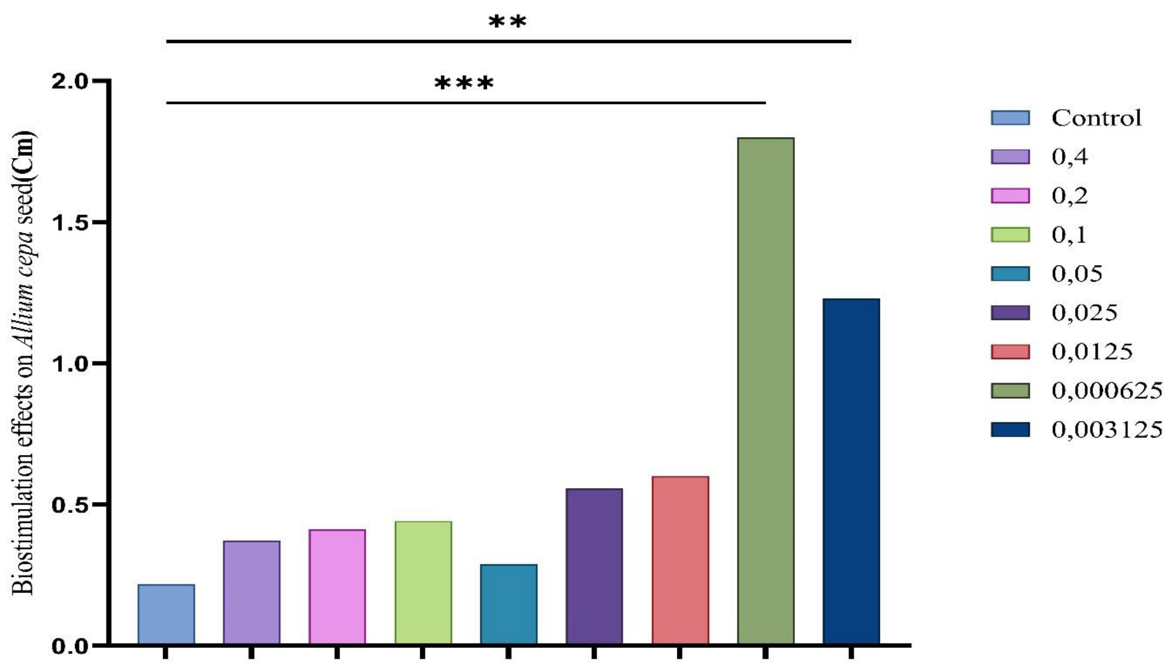

Figure 22. Tukey's Test Results of Biostimulation Effects on *Allium cepa* Seed Germination at Different Concentrations after 10 days.

The highest mean germination was observed at the concentration of 0.0125 (0.601 cm), followed by 0.003125 (1.23 cm), showing a moderate yet significant increase compared to the control group. The control group, with a mean of 0.217 cm, exhibited the least germination. Concentrations 0.4, 0.2, and 0.1 (with means of 0.372 cm, 0.412 cm, and 0.4425 cm, respectively) demonstrated a relatively similar but modest increase in germination compared to the control group, although these differences were not as significant as the higher concentrations **(Figure22)**.

Table 8. Mean of Biostimulation Effects on *Allium cepa* Seed Germination at Different Concentrations after 10 days.

	Control	**0,4**	**0,2**	**0,1**	**0,05**	**0,025**	**0,0125**	**0,000625**	**0,003125**
Mean	0,217	0,372	0,412	0,4425	0,290	0,557	0,601	1,8***	1,23**

Notably, the concentrations 0.00625 and 0.003125 exhibited significant differences in germination compared to the control group, with asterisks (** and ***) indicating a statistically significant difference ($p < 0.05$) according to the Tukey's test **(Table 8)**. The 0.0125 concentration showed the most significant stimulation, with a marked increase in germination, suggesting its potential as the optimal dose for promoting seed growth. In contrast, the lower concentrations (such as 0.05 and 0.025) showed moderate germination increases, but these differences were not statistically significant when compared to the control group **(Figure 23)**.

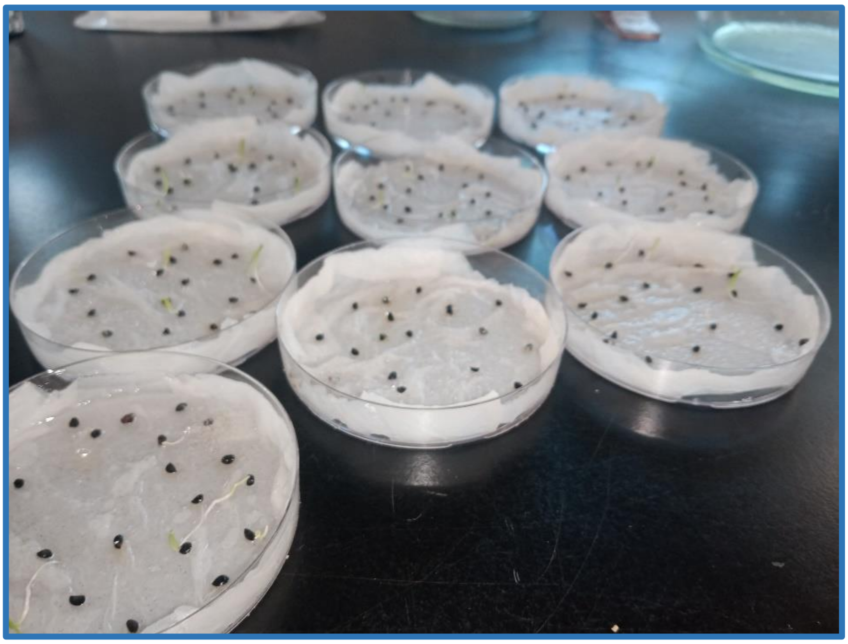

Figure 23. Hydroponic root stimulation trials **(personal photo).**

These results emphasize that higher concentrations, particularly around 0.0125, induce significant biostimulation in seed germination, while lower concentrations, though slightly more effective than the control, do not lead to the same level of growth. The statistical significance further confirms that the differences in germination observed between these groups are not due to random chance but are likely attributable to the effects of the concentration treatments.

The present hydroponic root stimulation trial demonstrated a clear concentration-dependent biostimulatory effect on *Allium cepa* seed germination and early seedling growth, with statistically significant differences confirmed via ANOVA (F = 7.06, $p < 0.000001$). The concentration of 0.0125 yielded the highest mean germination (0.601 cm), indicating an optimal dose beyond which further increases in concentration did not enhance growth significantly. These findings align closely with a growing body of literature emphasizing dose-dependent efficacy of various biostimulants, such as humic substances, seaweed extracts, protein hydrolysates, and amino acids, across multiple crops **(Li et al., 2022; Paul et al., 2019; Shahrajabian et al., 2021)**. Similar to the current study, these trials frequently identify low to moderate concentrations as the most effective, with benefits plateauing or diminishing at higher doses. For instance, seaweed extracts and protein hydrolysates exhibit maximal effects at 0.02–

0.05% concentrations in tomato and other vegetables **(Paul et al., 2019; Shahrajabian et al., 2021)**, mirroring the growth trends observed in *Allium cepa*. Moreover, the timing of application early in the developmental stage has been shown to be critical for maximizing biostimulant effectiveness **(Ying et al., 2022)**, supporting the 10-day observation window used in the present study. Although some studies employ repeated treatments, others demonstrate that single, early applications, as applied here, can significantly enhance germination and root development **(Rouphael & Colla, 2020; Shahrajabian et al., 2021)**. Importantly, while our experiment was conducted under controlled conditions, similar trends have been observed in field settings, with stronger responses noted in suboptimal environments **(Sleighter et al., 2023)**.

IV. Conclusion

IV.CONCLUSION

Nowadays, a large number of medicinal plants have very important pharmaco-biological properties which have many applications in various fields, including medicine, pharmacy, nutrition and agriculture.

The aim of our work is to evaluate the antioxidant, antimicrobial and anti-inflammatory activity in vitro of the ethanolic extract (Ethanol and distilled water ; 80% : 20% ; V/V) and chlorophyll and bio-stimulation test of *Ludwigia grandiflora*. In order to assess its phytochemical and biological properties, we studied the following parameters: maceration and extraction of a hydroalcoholic extract containing Ethanol/Distilled Water (80%: 20%, V/V), phytochemical screening to measure total phenols, flavonoids and tannins, study of antioxidant activity using three methods: DPPH, Total Antioxidant Capacity (TAC) and ABTS, Chlorophyll test (chlorophyll a , chlorophyll b), antimicrobial activity studies against 4 microorganisms GRAM - (*Escherichia coli and Pseudomonas aeruginosa)* and GRAM+ (*Staphylococcus aureus, Candida albicvans*) , anti-inflammatory activity using two methods (HSA, BSA) and the bio-stimulation procedure takes 10 days.

Hydroalcoholic maceration yielded an ethanolic extract of 7.8% of plant dry matter. Phytochemical screening of phenolic compounds showed values of 84.542±7.589mg EAG/g for total phenols, 34.080±0.384mg EQ/g for flavonoids and17.921±0.3468mg EAT/g for tannins.

Tests on the antioxidant activity of the ethanolic extract show good antioxidant activity with TAC values of 0.317±0.011 and DPPH of 0.495±0.09 mg/ml and Abts of 0.114±0.002 mg/ml.

The chlorophyll content measured 5.5370 mg/L for chlorophyll a, 3.2571 mg/L for chlorophyll b, and a total of 8.7941 mg/L demonstrates a high photosynthetic pigment concentration.

The results of the antibacterial activity of the ethanolic extract of *Ludwigia grandiflora* using the agar diffusion method showed very significant activity on various types of microorganism. *Staphylococcus aureus* was the most sensitive , reaching a maximum of 29±0.0 mm at 10 mg/mL, followed by *Candida albicans* suggesting good antifungal efficacy at high concentrations, there was a steady increase in activity, particularly marked between 1 and 7.5 mg/mL, than *Escherichia coli* the zone of inhibition increased steadily to reach 20±0.6 mm at the maximum concentration of 10 mg/mL, these results suggest a significant dose-dependent response, with notable antibacterial efficacy from 5.0 mg/mL. Finally *Pseudomonas aeruginosa* at 0.5 mg/mL, the zone of inhibition was 6.4±0.3 mm, rising to 19±0.0 mm at 10 mg/mL, indicating a lower sensitivity of the pathogen to this compound, which was the least affected. This ranking suggests that the compound may be better suited to treating Gram-positive and fungal infections, while requiring higher concentrations to act on resistant Gram-negative bacteria.

The ethanolic extract of *Ludwigia grandiflora* demonstrated notable anti-inflammatory activity through both hemolytic stabilization and protein denaturation assays. In the hemolytic stabilization assay, the extract exhibited a clear concentration-dependent inhibition of protein denaturation, with the percentage inhibition increasing from 16.30% at 100 mg/mL to 62.39% at 1000 mg/mL. This maximal effect slightly exceeded that of the reference anti-inflammatory drug, diclofenac sodium, which showed 60.87% inhibition at 100 mg/mL. Similarly, in the protein denaturation assay, the extract showed a progressive inhibition of heat-induced albumin denaturation, ranging from 16.67% at 100 mg/mL (corrected absorbance : 0.360) to 58.33% at 1000 mg/mL (corrected absorbance : 0.165), closely approaching the inhibitory effect of diclofenac sodium. The control sample (albumin only) exhibited the highest absorbance (0.460) and no inhibition, confirming the reliability of the assay. Taken together, these results indicate that *Ludwigia grandiflora* extract exerts a significant and dose-dependent anti-denaturation effect in both models, suggesting its potential as a natural anti-inflammatory agent comparable to conventional pharmaceutical drugs.

The bio-stimulation resuts showed that the highest mean germination was observed at 0.003125 (1.23 cm), followed by 0.0125 (0.601 cm), both significantly higher than the control group (0.217 cm). Other concentrations such as 0.1 (0.4425 cm), 0.2 (0.412 cm), and 0.4 (0.372 cm) showed modest increases compared to the control. These results suggest that lower concentrations of the extract exert a more effective biostimulatory effect on seed germination than higher concentrations or untreated seeds.

This study paves the way for future research into optimising the use of this plant in various fields for its pharmacological and agricultural potential, opening up prospects for phytotherapy and plant bio-stimulation. At the same time, extending studies on its antimicrobial action spectrum to include evaluation against a wider range of pathogenic micro-organisms would help to determine its versatility and therapeutic potential. Furthermore, the plant's bioactive compounds could offer valuable applications in the cosmetic industry, particularly for formulations targeting oxidative stress and microbial skin conditions. In a broader context, this research aligns with the principles of sustainable development by promoting the valorization of underutilized, local botanical resources for health, wellness, and industrial innovation.

V. Refrences

V. REFERENCES

V. Refrences

1. **Altir, N., Ali, A., Gaafar, A., Qahtan, A., Abdel-Salam, E., Alshameri, A., Hodhod, M., & Almunqedhi, B. (2021).** Phytochemical profile, in vitro antioxidant, and anti-protein denaturation activities of Curcuma longa L. rhizome and leaves. *Open Chemistry*, 19, 945 - 952. https://doi.org/10.1515/chem-2021-0086

2. **Arnon, D. I. (1949).** Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, *24*(1), 1–15. https://doi.org/10.1104/pp.24.1.1

3. **Aymard, P., Durand, D., et Nicolai, T. 1996**. The effect of temperature and ionic strength on the dimerisation of beta-lactoglobulin. Int J Biol Macromol, 19 : 213- 221.

4. **Barloy, D., Lemus, L., Krueger-Hadfield, S., Huteau, V., & Coriton, O. (2023).** Genomic relationships among diploid and polyploid species of the genusludwigial. sectionjussiaeausing a combination of molecular cytogenetic, morphological, and crossing investigations. https://doi.org/10.1101/2023.01.02.522458.

5. **Barloy, D., Lemus, L., Krueger-Hadfield, S., Huteau, V., & Coriton, O. (2024).** Genomic relationships among diploid and polyploid species of the genus *ludwigia* l. section jussiaea using a combination of molecular cytogenetic, morphological, and crossing investigations. Peer Community Journal, 4. https://doi.org/10.24072/pcjournal.364.

6. **Barloy-Hubler, F., Gac, A., Boury, C., Guichoux, E., & Barloy, D. (2023).** Sequencing, de novo assembly of *ludwigia* plastomes, and comparative analysis within the onagraceae family. https://doi.org/10.1101/2023.10.20.563230.

7. **El-Gawwas, E. and Soliman, M. (2023).** Effect of organic and biostimulants on yield and quality of evening primrose oil (oenothera biennis l.). Journal of Plant and Food Sciences, 0(0), 0-0. https://doi.org/10.21608/jpfs.2023.258502.1002.

8. **Farias, D., Funez, L., & Gasper, A. (2024).** Synopsis of onagraceae (myrtales) in the state of santa catarina, southern brazil. Rodriguésia, 75. https://doi.org/10.1590/2175-7860202475087.

9. **Sheidai, M., Rahimi, S., Mehrabian, A., & Koohdar, F. (2024)**. Looking for a daptivegeographical snpsin the genus *epilobium*. https://doi.org/10.21203/rs.3.rs-5429943/v1.

10. **Hussner, A., Windhaus, M., & Starfinger, U. (2016).** From weed biology to successful control : an example of successful management of *Ludwigia grandiflora* in germany. Weed Research, 56(6), 434-441. https://doi.org/10.1111/wre.12224.

V. REFERENCES

11. **Nehring, S. and Kolthoff, D. (2011).** The invasive water primrose *Ludwigia grandiflora* (michaux) greuter & burdet (spermatophyta : onagraceae) in germany : first record and ecological risk assessment. Aquatic Invasions, 6(1), 83-89. https://doi.org/10.3391/ai.2011.6.1.10.

12. **Billet, K., Genitoni, J., Bozec, M., Renault, D., & Barloy, D. (2018).** Aquatic and terrestrial morphotypes of the aquatic invasive plant, *Ludwigia grandiflora,* show distinct morphological and metabolomic responses. Ecology and Evolution, 8(5), 2568-2579. https://doi.org/10.1002/ece3.3848.

13. **Blois, M.S. 1958**. Antioxidant determinations by the use of a stable free radical, Nature, 181: 1199-1200.

14. **Boualam, K., Ndiaye, B., Harhar, H., Tabyaoui, M., Ayessou, N., & Taghzouti, K. (2021)**. Study of the Phytochemical Composition, the Antioxidant and the Anti-Inflammatory Effects of Two Sub-Saharan Plants: Piliostigma reticulatum and Piliostigma thonningii. *Advances in Pharmacological and Pharmaceutical Sciences*, 2021. https://doi.org/10.1155/2021/5549478

15. **Bozin B., Mimica-Dukic N. et Samojlik I. (2008).** Phenolics as antioxidants in garlic (*Allium sativum L., Alliaceae*). Food Chemistry., 111:925–929.

16. **Chaiyana, W., Anuchapreeda, S., Leelapornpisid, P., Phongpradist, R., Viernstein, H., and Mueller, M. 2016.** Development of Micro emulsion Delivery System of Essential Oil from Zingiber cassumunar Roxb. Rhizome for Improvement of Stability and Anti-Inflammatory Activity. AAPS PharmSciTech, 18 : 1332–1342.

17. **Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002).** Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178-182.

18. **Cheurfa M., Allem R., Sebaihia M., Belhireche S. (2013).** Effet de l'huile essentielle de Thymus Vulgaris les bactéries pathogènes sur responsables de gastroentérites. Phytothérapie,11(3), 154-160.

19. **Chirinos J., Leal A. and Montilla J. (2006)** Use Alternative Biological Inputs for Sustainable Agriculture in the South of Anzoategui state. Applied and Interdisciplinary Sciences, Biotechnology. Digital Magazine Ceniap Today, 11, 1-7.

20. **Das, K., Asdaq, S., Khan, M., Amrutha, S., Alamri, A., Alhomrani, M., Alsanie, W., Bhaskar, A., Shree, G., & Harshitha, P. (2022).** Phytochemical investigation and evaluation of invitro anti-inflammatory activity of Euphorbia hirta ethanol leaf and root extracts: A comparative study. *Journal of King Saud University - Science*. https://doi.org/10.1016/j.jksus.2022.102261

21. **Garcia-Ruiz A., Bartolomé B., Martinez-Rodriguez A.J., Pueyo E., Martin-Alvarez P.J., and Moreno-Arribas M.V. (2008)**. Potential of phenolic compounds for controlling lactic acid bacteria growth in wine. Food Control. 19: 835–841.

22. **Graversen, P., Østergaard, L., Voldstedlund, M., Wandall-Holm, M., Smerup, M., Køber, L., … & Fosbøl, E. (2023).** Microbiological etiology in patients with ie undergoing surgery and for patients with medical treatment only : a nationwide study from 2010 to 2020. Microorganisms, 11(10), 2403. https://doi.org/10.3390/microorganisms11102403.

23. **Graversen, P., Østergaard, L., Voldstedlund, M., Wandall-Holm, M., Smerup, M., Køber, L., … & Fosbøl, E. (2023).** Microbiological etiology in patients with ie undergoing surgery during admission and for patients with medical treatment only : a nationwide study from 2010 to 2020. https://doi.org/10.21203/rs.3.rs-3079711/v1.

24. **Habibur, Rahman., Chinna Eswaraiah, M., Vakati, K., and Madhavi, P. 2012**. In-vitro studies suggest probable mechanism of Eucalyptus oil for anti-inflammatory and antiarthritic activity. International Journal of Phytopharmacy, 2(3) : 81-83.

25. **Hamel T., & Saci A.,2024.** *Ludwigia grandiflora* (Michx.) Greuter & Burdet, a new addition to the allochthonous flora of Algeria and first report in Mediterranean Africa, Bulletin de la Société Vaudoise des Sciences Naturelles 103: xx-xx. international acronym GdB, http://gdebelair.com/

26. **Hamion, G., Aucher, W., Tardif, C., Miranda, J., Rouger, C., Imbert, C., … & Girardot, M. (2022).** Valorization of invasive plant extracts against the bispecies biofilm *staphylococcus aureus–candida albicans* by a bioguided molecular networking screening. Antibiotics, 11(11), 1595. https://doi.org/10.3390/antibiotics11111595.

27. **Thouvenot, L., Haury, J., & Thiébaut, G. (2013).** A success story : water primroses, aquatic plant pests. Aquatic Conservation Marine and Freshwater Ecosystems, 23(5), 790-803. https://doi.org/10.1002/aqc.2387.

28. **Hassan, N., Alrawy, M., Abdelrahman, M., Gad, E., & Shafik, N. (2023).** Molecular detection of efflux pump and virulence factors genes in *pseudomonas aeruginosa*. Microbes and Infectious Diseases, 0(0), 0-0. https://doi.org/10.21608/mid.2023.211752.1521.

29. **Hussner, A., Windhaus, M., & Starfinger, U. (2016).** From weed biology to successful control : an example of successful management of *Ludwigia grandiflora* in germany. Weed Research, 56(6), 434-441. https://doi.org/10.1111/wre.12224.

V. REFERENCES

30. **Imen Smida, Alaa Sweidan, Yasmine Souissi, Isabelle Rouaud, Aurélie Sauvager, et al. Anti-Acne, Antioxidant and Cytotoxic Properties of *Ludwigia peploides* Leaf Extract. International Journal of Pharmacognosy and Phytochemical Research, 2018**, 10 (7), pp.271-278. ffhal-01928736ff.

31. **İpek, S., Şahin, A., Güngör, Ş., Yurttutan, S., Güllü, U., Inal, S., … & Güllü, Ş. (2022).** Nosocomial infections in non-covid-19 pediatric patients prior to and during the pandemic in a pediatric intensive care unit. Cureus.

32. **Kandikattu K, Bharath Rathna Kumar P, Venu Priya R, Sunil Kumar K, Ranjith Singh.B.Rathore. evaluation of anti-inflammatory activity of canthium parviflorum by *in-vitro* method. Indian Journal of Research in Pharmacy and Biotechnology 2013**; 1(5): 729-730.

33. **Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004).** Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2(2), 123-140.

34. **Kato-Noguchi, H. and Kato, M. (2024).** The allelopathy of the invasive plant species *Ludwigia* decurrens against rice and paddy weeds. Agriculture, 14(8), 1297. https://doi.org/10.3390/agriculture14081297.

35. **Khadka, S., Sherchand, J., Pokhrel, B., Parajuli, K., Mishra, S., Sharma, S., … & Rijal, B. (2017).** Isolation, speciation and antifungal susceptibility testing of *candida* isolates from various clinical specimens at a tertiary care hospital, nepal. BMC Research Notes, 10(1). https://doi.org/10.1186/s13104-017-2547-3 .

36. **Koffi ̧E., Sea., Dodehe Y. Soro S. (2010)**. Effect of solvent type on extraction of polyphenolsfrom twenty-three Ivorian plants. Journal of Animal & Plant Sciences, 2010.

37. **Ladoh Yemeda, C. F., Dibon S. D., Nyegue, M. A., Djembissi Talla, R. P., Lenta Ndjakou, B., Mpondo, E., Yinyang, J., Wansi, J. D. 2014**. Activité antioxydante des extraits méthanoliques de Phragmanthera capitata (Loranthaceae) récoltée sur Citrus sinensis. Journal of applied Bioscience. 84 : 7636-7643.

38. **Lewis, M. and Williams, D. (2017).** Diagnosis and management of oral candidosis. BDJ, 223(9), 675-681. https://doi.org/10.1038/sj.bdj.2017.886 .

39. **Li Y, et al. (2007).** An enzyme-coupled assay for amido-transferase activity of glucosamine-6-phosphate synthase. Anal Biochem 370(2) :142-6.

40. **Li, J., Van Gerrewey, T., & Geelen, D. (2022).** A Meta-Analysis of Biostimulant Yield Effectiveness in Field Trials. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.836702

41. Liguori, L., Adiletta, G., Nazzaro, F., Fratianni, F., Marisa, D., & Albanese, D. (2019). Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the mediterranean area. Journal of Food Measurement & Characterization, 13(2), 1232-1241. https://doi.org/10.1007/s11694-019-00038-2.

Liu, S., Hoch, P., Diazgranados, M., Raven, P., & Barber, J. (2017). Multi-locus phylogeny of *ludwigia* (onagraceae) : insights on infra- generic relationships and the current classification of the genus. Taxon, 66(5), 1112-1127. https://doi.org/10.12705/665.7.

42. Marrelli, M., Amodeo, V., Puntillo, D., Statti, G., & Conforti, F. (2022). In vitro antioxidant and anti-denaturation effects of Buglossoides purpurocaerulea (L.) I. M. Johnst. fruit extract. *Natural Product Research*, 37, 1012 - 1015. https://doi.org/10.1080/14786419.2022.2096607

43. Mikulyuk, A. (2009). *Ludwigia grandiflora* (water primrose). https://doi.org/10.1079/cabicompendium.109148.

44. Mizushima, Y., and Kobayashi, M. 1968. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J. Pharm.Pharmac, 20 : 169-173.

45. Nasution, A. (2013). Virulence factor and pathogenicity of candida albicans in oral candidiasis. World Journal of Dentistry, 4(4), 267-271. https://doi.org/10.5005/jp-journals-10015-1243 .

46. Ngameni B., Kuete V., Simo I.K., Mbaveng A.T., Awoussong P.K., Patnam R., Roy R. and Ngadjui B.T., (2009). Antibacterial and antifungal activities of the crude extract and compounds from *Dorstenia turbinata* (Moraceae). South African J Botany. 75 : 256-261.

47. Ngoua-Meye-Misso, R., Sima-Obiang, C., De La Croix Ndong, J., Ondo, J., Abessolo, O., & Obame-Engonga, L. (2018). Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of Lophira procera A. Chev. (Ochnaceae) medicinal plant from Gabon. *Egyptian Journal of Basic and Applied Sciences*, 5, 80 - 86. https://doi.org/10.1016/j.ejbas.2017.11.003

48. Parin, ©., Ugur, U., Sukru, K., Özge, O., Tugba, Y., & Uğur, P. (2018). Untitled. Approaches in Poultry Dairy & Veterinary Sciences, 3(4). https://doi.org/10.31031/apdv.3.4.

49. Patel, M. (2022). Oral cavity and *candida albicans* : colonisation to the development of infection. Pathogens, 11(3), 335. https://doi.org/10.3390/pathogens11030335.

V. REFERENCES

50. **Paul, K., Sorrentino, M., Lucini, L., Rouphael, Y., Cardarelli, M., Bonini, P., Reynaud, H., Canaguier, R., Trtílek, M., Panzarová, K., & Colla, G. (2019)**. Understanding the Biostimulant Action of Vegetal-Derived Protein Hydrolysates by High-Throughput Plant Phenotyping and Metabolomics: A Case Study on Tomato. *Frontiers in Plant Science*, 10. https://doi.org/10.3389/fpls.2019.00047

51. **Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978).** A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of agricultural and food chemistry*, 26(5), 1214-1218.

52. **Pysek P., Pergl J., Essl F., Lenzner B., Dawson W., Kreft H., Weigelt P., Winter W., Kartesz J. & Nishino M., 2017**. Naturalized alien flora of the world : species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. Preslia 89 (3) : 203-274. https://doi.org/10.23855/preslia.2017.203.

53. **Rajalakshmi, R., Mukeshbabu, N., Doss, A., Priya, R., Rani, T., Pole, R., & Satheesh, S. (2024).** Phytochemical screening and in vitro anti-inflammatory properties of Jatropha maheshwarii Subram. & Nayar – an endemic plant. *Pharmacological Research - Natural Products*. https://doi.org/10.1016/j.prenap.2024.100058

54. **Ramalakshmi K., Rahath Kubra I. et Jagan Mohan Rao L. (2008)**. Antioxidant potential of low-grade coffee beans. Food Research International. 41: 96–103.

55. **Re, R., Pellegrini, N., Proteggente, A., Pannala, A.,Yang, M., Rice-Evans, C. (1999)**. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.,* 26, 1231.

56. **Richardson D.M., Pysek P., Rejmanek M., Barbour MG., Panetta F.D. & West C.J. 2000**. Naturalization and invasion of alien plants: concepts and definitions. Diversity & Distributions 6 :93 107. doi :10.1046/j.1472-4642.2000.00083. x.

57. **Romani A, et al. (2006).** Analysis of condensed and hydrolysable tannins from commercial plant extracts. J Pharm Biomed Anal 41(2) :415-20.

58. **Rouphael, Y., & Colla, G. (2020).** Toward a Sustainable Agriculture Through Plant Biostimulants: From Experimental Data to Practical Applications. *Agronomy*. https://doi.org/10.3390/agronomy10101461

59. **Shaaban, M., Emam, S., & Ramadan, D. (2019).** Molecular detection of virulence genes in *pseudomonas aeruginosa* isolated from patients with burn wound infections from burn and plastic surgery department at benha teaching hospital. Egyptian Journal of Medical Microbiology, 28(4), 27-32.

V. REFERENCES

60. **Shahrajabian, M., Chaski, C., Polyzos, N., & Petropoulos, S. (2021).** Biostimulants Application: A Low Input Cropping Management Tool for Sustainable Farming of Vegetables. *Biomolecules*, 11. https://doi.org/10.3390/biom11050698

61. **Sleighter, R., Hanson, T., Holden, D., & Richards, K. (2023).** Abiotic Stress Mitigation: A Case Study from 21 Trials Using a Natural Organic Matter Based Biostimulant across Multiple Geographies. *Agronomy*. https://doi.org/10.3390/agronomy13030728

62. **Thouvenot, L., Haury, J., & Thiébaut, G. (2013).** A success story: water primroses, aquatic plant pests. Aquatic Conservation Marine and Freshwater Ecosystems, 23(5), 790-803. https://doi.org/10.1002/aqc.2387.

63. **Tona, M., Tareq, A., Sayeed, M., Mahmud, M., Jahan, I., Sakib, S., Shima, M., & Emran, T. (2020).** Phytochemical screening and in vitro pharmacological activities of methanolic leaves extract of Caryota mitis., 3, 109. https://doi.org/10.5455/jabet.2020.d114

64. **Tóth, V. R., Villa, P., Pinardi, M., & Bresciani, M. (2019).** Aspects of invasiveness of *Ludwigia* and *Nelumbo* in shallow temperate fluvial lakes. *Frontiers in Plant Science*, 10, 647. https://doi.org/10.3389/fpls.2019.00647 .

65. **Trobisch, A., Schweintzger, N., Kohlfürst, D., Sagmeister, M., Sperl, M., Grisold, A., … & Zenz, W. (2022).** Osteoarticular infections in pediatric hospitals in europe : a prospective cohort study from the euclids consortium. Frontiers in Pediatrics, 10. https://doi.org/10.3389/fped.2022.744182.

66. **Ventrella, M.C., Marinho, C.R. 2008**. Morphology and histochemistry of glandular trichomes of Cordia verbenaceae (Boraginaceae) leaves. Revista Brasilia Botanica 457–467. Vol. 5, Issue 3: 550- 558.

67. **Wang, H., Yang, H., Yu, X., Xie, Y., Bai, Y., Dai, Q., … & Tang, Y. (2024).** Biological features and quality comprehensive analysis of twelve germplasm resources of the genus *allium* from tibet. Frontiers in Plant Science, 15. https://doi.org/10.3389/fpls.2024.1393402.

68. **Ying M., Freitas, H., & Dias, M. (2022**). Strategies and prospects for biostimulants to alleviate abiotic stress in plants. *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.1024243

69. **Zhao, H., Guo, T., Zhou, Y., Zhao, F., Sun, Y., Wang, Y., … & Hao, Y. (2025).** Major causative bacteria of dairy cow mastitis in the inner mongolia autonomous region, china, 2015–2024 : an epidemiologic survey and analysis. Veterinary Sciences, 12(3), 197. https://doi.org/10.3390/vetsci12030197.

70. **Zhao, X., Lin, F., Li, H., Li, H., Wu, D., Geng, F., … & Gan, R. (2021).** Recent advances in bioactive compounds, health functions, and safety concerns of onion (*allium cepa* l.). Frontiers in Nutrition, 8. https://doi.org/10.3389/fnut.2021.669805.

Webography

71. **Anonymous 1.** https://fuedei.org/en/ludwigia-grandiflora-ssp-hexapetala-water-primrose/. Consulter le 10/06/2025.

72. **Anonymous 2.** https://www.pasteur.fr/fr/journal-recherche/dossiers/salmonellose-institut-pasteur-lance-alerte. Consulter le 10/06/2025.

73. **Anonymous 3.** https://www.sciencephoto.com/media/874035/view/staphylococcus-aureus-sem. Consulter le 10/06/2025.

74. **Anonymous 4.** https://www.sciencephoto.com/media/1003489/view/pseudomonas-aeruginosa-sem. Consulter le 10/06/2025.

VI. Appendices

APPENDIX 1

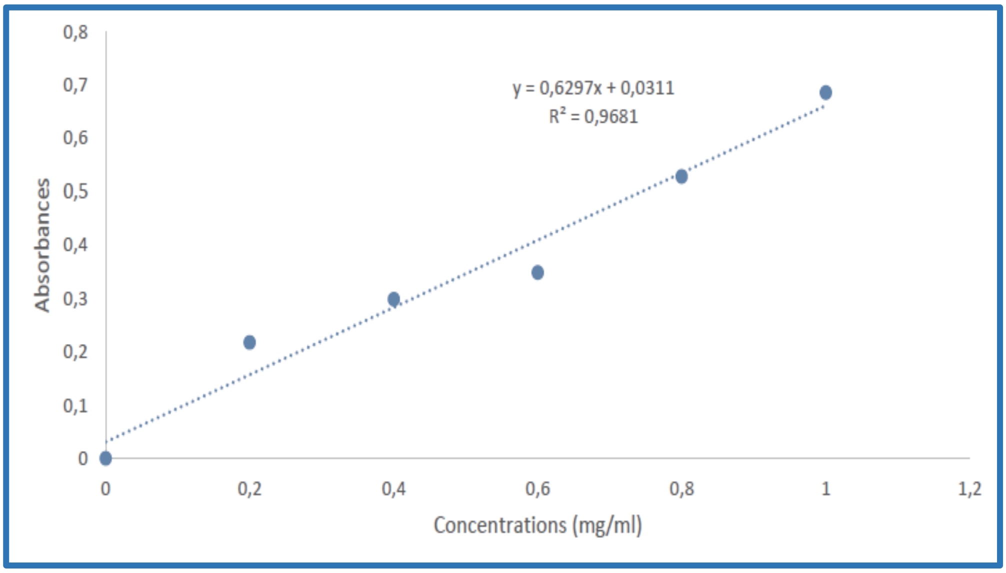

Figure 14. Gallic acid calibration curve for the determination of total phenols

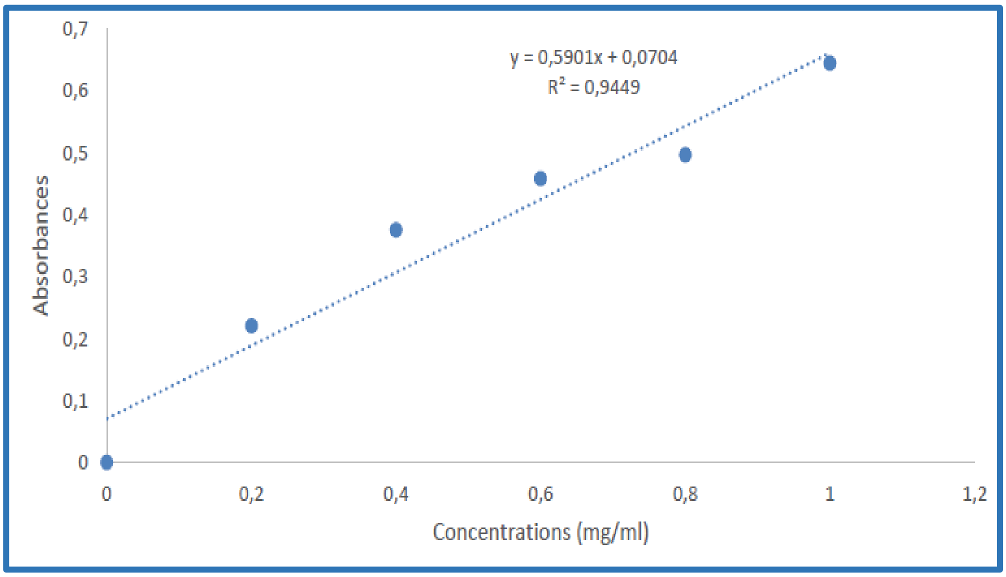

Figure 15. Calibration curve for quercetin flavonoid assay.

APPENDIX 2

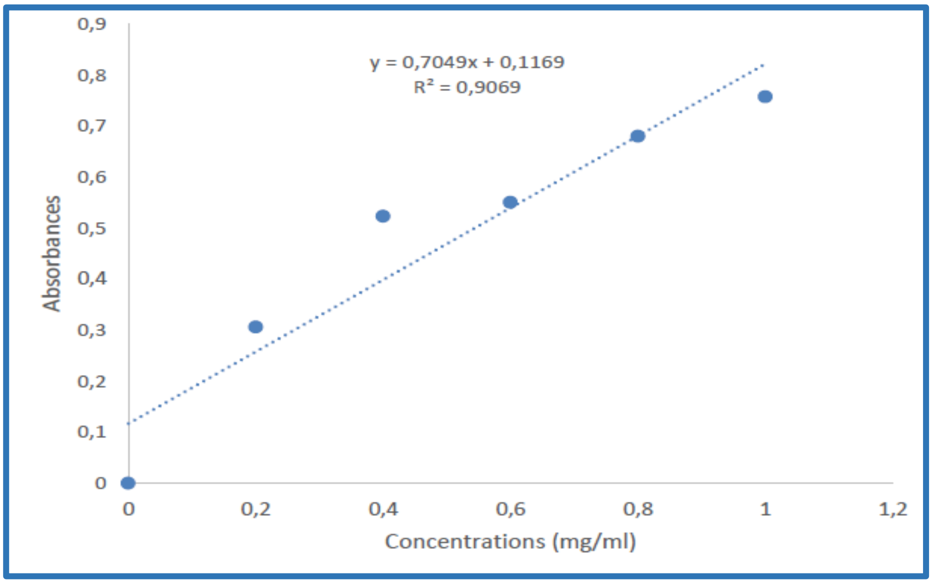

Figure 16. Tannic acid calibration curve for tannin determination.

Full name : Chebel Roukaya

Full name : Doghmane Lamisse Nourhane

Title : Study of the phenolic compounds, biostimulation and biological activities of *Ludwigia grandiflora* (Michx.) Greuter & Burdet, 1987

Abstract : *Ludwigia grandiflora* (Michx.) Greuter & Burdet, 1987, a member of the Onagraceae family, has garnered significant attention for its phytochemical richness and therapeutic potential. This study investigates the bioactive compounds present in the ethanolic extract of *L. grandiflora*, focusing on its antioxidant, antimicrobial, and anti-inflammatory activities. Using a comprehensive set of in vitro assays, including DPPH, ABTS, and total antioxidant activity tests, the extract demonstrated potent free radical scavenging activity and high antioxidant capacity, with an IC_{50} of 0.495 mg/mL for DPPH. Antimicrobial evaluation revealed significant inhibitory effects on both Gram-negative and Gram-positive bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, with the most prominent antibacterial activity observed against *S. aureus*. Additionally, the extract exhibited substantial anti-inflammatory effects, as evidenced by its ability to inhibit protein denaturation in both human and bovine serum albumin assays, showing up to 62.39% inhibition at the highest concentration.Phytochemical analysis revealed high concentrations of phenolic compounds, flavonoids, and tannins, which are likely contributors to the observed bioactivities. Chlorophyll content was also assessed, revealing high levels of chlorophyll a (5.5370 mg/L), chlorophyll b (3.2571 mg/L), and total chlorophyll (8.7941 mg/L), indicating a strong presence of photosynthetic pigments. Furthermore, biostimulation assays showed that low concentrations of the extract significantly enhanced seed germination, with the highest germination rate observed at 0.003125 (1.23 cm), followed by 0.0125 (0.601 cm), both outperforming the control group. These results suggest that *L. grandiflora* holds significant promise as a source of natural antioxidants, antimicrobial agents, anti-inflammatory substances, and plant biostimulants, offering potential for therapeutic applications in traditional medicine, pharmaceutical development, and sustainable agriculture.

Key words : *Ludwigia grandiflora* (Michx.) Greuter & Burdet, 1987, antioxidant activity, antimicrobial activity, phenolic compounds, anti-inflammatory activity, biostimulation.

Academic Year : 2024/2025