ALGERIAN PEOPLE'S DEMOCRATIC REPUBLIC
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
UNIVERSITE YAHIA FARES DE MEDEA



Faculty of Sciences
Field: Natural and Life Sciences
Specialization: Applied Microbiology

Master's Thesis

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For obtaining a master's degree in Natural and Life Sciences

Specialization: Applied Microbiology

Theme

CONTRIBUTION TO THE VALORIZATION OF THE ANTIMICROBIAL EFFECTS OF PLANT-DERIVED BIOMOLECULES FROM THE ARTEMISIA ALOE VERA , BROCCHIA CENIREA , AND MORINGA

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Academic Year 2022-2023

Gratitude

I am deeply grateful to God for the strength and determination to overcome challenges and achieve this work. With a heart full of gratitude, I sincerely thank God for His blessings and constant presence in my life .

This work is the result of numerous collaborations, and it is my pleasure to express my heartfelt thanks to all those who have directly or indirectly contributed to its realization.

- Mr. Mahdjoubi Djilali, who honored me by supervising this work, I extend my sincere thanks to him for his assistance, guidance, and valuable advice, which have been of great value.
- To the members of the jury who graciously agreed to preside over this committee, I express my gratitude for their generosity and availability in examining this work.

- I want to dedicate this achievement to both of my parents To **my incredible Mom**, I am immensely grateful for your invaluable support and guidance throughout this work This accomplishment is as much yours as it is mine. I couldn't have done it without your patience, expertise, and unwavering belief in my abilities.
- To **my Dad** , Thank you for always being there, ready to lend a helping hand whenever I needed it. Your belief in my abilities and your constant motivation have pushed me to go above and beyond.
- To **Youcef DAHMANIi** I wanted to take a moment to express my sincere gratitude for your invaluable support and guidance throughout this work unwavering presence have made a significant impact on my journey. Thank you for being a constant source of inspiration .
- To **Zola** I want to express my deepest gratitude for the incredible support and assistance you have provided me with . Your kindness, patience, and guidance have been invaluable to me. Whenever I needed a helping hand you were always there, ready to provide the support I needed.
- To Mr BELHAFRI , we express our heartfelt thanks to the teacher for his invaluable contribution and for helping us in the rabbit experiment .

We would like to express our gratitude to the entire team of SAIDAL (Medea) who warmly welcomed us, including Mr. Mohamed Amar, Mr. Karim Laidi, Ms. Sarah, Ms. Toumi, and Ms. Gumana. We are thankful for their encouragement, availability, assistance, and valuable advice. It has been an honor for us to work with them.

We would also like to thank Mr. kairaouni , Mr rahal Mohamed for their help.

Dedicace

I want to dedicate this achievement to both of ***my parents*** *It is a testament to your love, guidance, and unwavering support. Your encouragement, and sacrifices have been the pillars that held me up during the highs and lows,. Thank you for being my rock and my inspiration. I could not have come this far without you by my side. I will forever treasure the love you have given me and the invaluable lessons you have taught me. You are the source of my strength and the reason for my success I will forever be thankfull for both of you .also I want to dedicate this achievement to my brothers Aymen and Moatez and my beautiful sister Rimasse thank you for always being there for me .*

To my uncle Youcef DAHMANI: *I am truly fortunate to have you as my uncle and mentor. Your dedication and passion inspire me to reach for the highest goals and to never settle for mediocrity. Thank you for being a constant source of inspiration and for your unwavering support. Your guidance has made a profound impact on my personal and professional growth, and I am deeply grateful for your presence in my life.*

To Zola: *You have not only been an uncle's wife to me, but a mentor and a role model. Your strength, resilience, and determination have shown me the true meaning of perseverance and hard work. Your kindness and generosity have made a lasting impact, and I am truly blessed to have you in my life, your contributions to my life and my success are immeasurable, and I am forever grateful for your presence and guidance.*

To my dearest best friend Amina: Words *cannot express the depth of my gratitude for your unwavering support and constant presence in my life. You have been my rock, my confidant, and my guiding light through every twist and turn. Thank you for always believing in me, even when I doubted myself. Your encouragement and belief in my abilities have given me the strength to overcome challenges and reach for the stars. I am eternally grateful for your friendship.having you by my side is a constant source of comfort and reassurance.*

To my partener Radia : *To my amazing binome, thank you for your incredible collaboration and support. Our partnership has been a true testament to teamwork and shared goals." we have accomplished great things,*

To my adorable cousins Sirin and Aya *who have always been there for me and who have supported and encouraged me.*

To my dearest best friend fadou : *Thank you, my dear friend, for being the incredible person that you are and for always being there for me. Our bond is unbreakable, and I am truly blessed to have you in my life.*

To my grand parent who have always provided me with support and comfort and all my anculs and anties .To all my friends zola nouha yassmin, who have always provided me with support and comfort.

Fadoua

Dedicace

I dedicate this work To

my dear mother, you are the source of light that illuminates my life, the one who gave me birth and has been by my side at every step of my existence. Your unconditional love has been my refuge, and your strength and perseverance are an endless source of inspiration. You are the one who nurtured my dreams and encouraged me to pursue my passions. I love you.

To my father, You are my hero, my supporter at every stage of my life. You have been my role model of strength, integrity, and wisdom. I thank you from the depths of my heart for your love, selfless sacrifices, and everything you have done for me and our family.

To my brother, My life companion, my friend, my confidant from our childhood until now. You have been a precious presence in my life, and I am proud to have a brother like you.

To my dear sisters, Marwa, Zineb, Kawthar, this dedication reminds you how special you are in my eyes. I will always be there for you.

To my wonderful partner "Fadoua", Our relationship is special, based on trust, understanding, and mutual respect. Together, we have formed an unbeatable team, overcoming obstacles with determination and mutual support. Your intelligence, creativity, and unwavering support inspire me to reach new heights. May this dedication remind you how much I love you and how much I value our relationship. Together, we are capable of great things.

To my precious friends, "Dounia, Khadidja, Zola, Amina," I am grateful to have such exceptional friends like you.

"Radhia"

Abstract

Our work consists of studying the biological activities of some medicinal plants by evaluation their antimicrobial, wound healing, burn, as well as their content of chemical groups and families. then we develop two different creams the first one based on the pure gel of aloe vera and the second one based on the hydroethanolic extract of aloe vera, we have chosen the following medicinal plant: aloe vera, Artemisia herba alba, moringa oleifera, brocchia cenirea.

The first step of our work involves conducting methanolic, aqueous n hydroethanolic extractions from the leaves of the following plants: moringa oleifera, Artemisia herba alba, brocchia cenirea. additionally, we performed an extraction of essential oils from the plants brocchia cenirea, Artemisia herbalba.

The second part involves conducting a phytochemical study, we have discovered the presence of these four plants containing those compounds (flavonoids, anthocyans, quinons, mucilages, tannins, glucosids, alkaloids) by using chemical substances and the results was determined through a change in the color of the plant, each of the four plants showed a different result.

The third part concerns the evaluation of the animicrobial and antifungal activity of all methanolic and aqueous and hyroethanolic extracts, as wellas the essential oils of two plants (*Brocchia cenirea ,Artemisia herba alba)*. then the determination of the irminimum inhibitory concentration (MIC) was carried out. we observed different results, some extracts exhibited strong activity while others showedweak activity. additionally, the essential oils of (brocchia cenirea, artemisia herba alba) exhibited strong activity which was confimed by the results obtained through the agar diffusion method.

The fourth part we focused on formulating two creams specifically forthe treatment of burns and wound scars based on aloe vera plant that is known for its therapeutic benefits.

Finally, the application of the first cream based on hydroethanolic extract of aloe vera plant which we developed reduced the burn area by 95% in rabbits, additionally the second cream formulated with the pure gel of aloe vera, we observed completely healing of the wound scar area in rabbits reducing it by 100% in 12 days. In conclusion, this study supports the use of these medicinal plants in the fiels of phytotherapy.

Key words: Artemisia herba alba, aloe vera, moringa oleifera, brocchia cenirea, antimicrobial activity, antifungal activity, flavonoids, anthocyans, quinons, mucilage, tannins, glucosids, alkaloids, cream, burn, wound scar .

Résumé

Notre travail consiste à étudier les activités biologiques de certaines plantes médicinales en évaluant leur activité antimicrobienne, leur capacité de cicatrisation des plaies et des brûlures, ainsi que leur contenu en groupes et familles de composés chimiques. Ensuite, nous développons deux crèmes différentes : la première à base de gel pur d'aloe vera et la deuxième à base d'extrait hydroéthanolique d'aloe vera. Nous avons choisi les plantes médicinales suivantes : *l'aloe vera, l'Artemisia herba alba*, le *moringa oleifera* et la *brocchia cenirea*.

La première étape de notre travail consiste à réaliser des extractions méthanoliques, aqueuses et hydroéthanoliques à partir des feuilles des plantes suivantes : le moringa oleifera, l'Artemisia herba alba et la brocchia cenirea. De plus, nous avons effectué une extraction d'huiles essentielles à partir des plantes brocchia cenirea et Artemisia herbalba.

La deuxième partie consiste à mener une étude phytochimique, au cours de laquelle nous avons découvert la présence de ces quatre plantes contenant les composés suivants (flavonoïdes, anthocyanes, quinones, mucilages, tanins, glucosides, alcaloïdes) en utilisant des substances chimiques, et les résultats ont été déterminés par un changement de couleur de la plante, chacune des quatre plantes présentant des résultats différents.

La troisième partie concerne l'évaluation de l'activité antimicrobienne et antifongique de tous les extraits méthanoliques, aqueux et hydroéthanoliques, ainsi que des huiles essentielles de deux plantes (brocchia cenirea, Artemisia herba alba). Ensuite, la détermination de la concentration inhibitrice minimale (CIM) a été réalisée. Nous avons observé des résultats différents, certains extraits présentant une forte activité tandis que d'autres montraient une faible activité. De plus, les huiles essentielles de brocchia cenirea et d'Artemisia herba alba ont présenté une forte activité, confirmée par les résultats obtenus par la méthode de diffusion sur agar.

La quatrième partie de notre étude a été axée sur la formulation de deux crèmes spécifiquement destinées au traitement des brûlures et des cicatrices de plaies, à base de la plante d'aloe vera reconnue pour ses bienfaits thérapeutiques.

Enfin, l'application de la première crème à base d'extrait hydroéthanolique de l'aloe vera que nous avons développée a réduit la zone de brûlure de 95% chez les lapins. De plus, la seconde crème formulée avec le gel pur d'aloe vera a permis une guérison complète de la zone de cicatrice de plaie chez les lapins, réduisant celle-ci de 100% en 12 jours. En conclusion, cette étude confirme l'utilisation de ces plantes médicinales dans le domaine de la phytothérapie.

Mots clés : *Artemisia herba alba*, *aloe vera*, *moringa oleifera*, *brocchia cenirea*, activité antimicrobienne, activité antifongique, flavonoïdes, anthocyanes, quinones, mucilage, tanins, glucosides, alcaloïdes, crème, brûlure, cicatrice de plaie.

ملخص

عملنا يتكون من دراسة الأنشطة البيولوجية لبعض النباتات الطبية من خلال تقييم تأثيرها المضاد للميكروبات وشفاء الجروحوالحروق، وكذلك تحديد محتواها من المجموعات الكيميائية والعائلات الكيميائية. ثم قمنا بتطوير كريمين مختلفين، الأول يعتمدعلى الجل النقي لنبات الألوة فيرا والثاني يعتمد على استخراج الألوة فيرا بالهيدروإيثانول. اخترنا النباتات الطبية التالية.

Aloe vera, Brocchia Cenirea, Artemisia herba alba, brocchia cenirea

الخطوة الأولى في عملنا تتضمن إجراء استخراجات ميثانولية ومائية وهيدروإيثانولية من أوراق النباتات التالية Moringa oleifera، aloe vera , brocchia cenirea Artemisia herba alba. بالإضافة إلى ذلك، قمنا بأخذ استخراج للزيوت العطرية من نباتات brocchia cenirea , Artemisia herba alba

الجزء الثاني يتضمن إجراء دراسة فيتوكيميائية، حيث اكتشفنا وجود هذه النباتات الأربعة التي تحتوي على المركبات التالية (الفلافونويدات، الأنثوسيانين، الكينونات، المواد اللزجة، التانينات، الجلوكوسيدات، القلويدات) باستخدام المواد الكيميائية وتم تحديد النتائج من خلال تغير لون النبات، حيث أظهرت كل من النباتات الأربعة نتائج مختلفة.

الجزء الثالث يتعلق بتقييم النشاط المضاد للميكروبات والفطريات لجميع الاستخراجات الميثانولية والمائية والهيدروإيثانولية، بالإضافة إلى الزيوت العطرية لنباتين (بروكيا سينيريا، آرتيميزيا هيربا البيضاء). تم إجراء تحديد التركيز الحد الأدنى المثبط .(MIC) لاحظنا نتائج مختلفة، حيث أظهرت بعض الاستخراجات نشاطًا قويًا بينما أظهرت البعض الآخر نشاطًا ضعيفًا. بالإضافة إلى ذلك، أظهرت الزيوت العطرية لـ (بروكيا سينيريا، آرتيميزيا هيربا البيضاء) نشاطًا قويًا تم تأكيده من خلال النتائج التي تم الحصول عليها من خلال طريقة انتشار الآجار.

الجزء الرابع تركزنا فيه على تصنيع كريمين خاصين لعلاج الحروق وندوب الجروح بناءً على نبات الألوة فيرا المعروف بفوائده العلاجية. في النهاية، أدى استخدام الكريم الأول الذي يعتمد على استخراج الألوة فيرا بالهيدروإيثانول إلى تقليل مساحة الحرق بنسبة 95٪ في الأرانب. بالإضافة إلى ذلك، لاحظنا تمام شفاء منطقة ندبة الجروح في الأرانب بنسبة 100٪ في 12 يومًا باستخدام الكريم الثاني الذي يعتمد على الجل النقي لنبات الألوة فيرا. في الختام، يدعم هذا الدراسة استخدام هذه النباتات الطبية في مجال العلاج النباتي.

الكلمات المفتاحية: بروكيا سينيريا، آرتيميزيا هيربا البيضاء، الويفيرا، مورينجا
مضاد للبكتيريا، مضاد للفطريات، فلافونوييدات،انثوسيانين، كينونات، مخاط ، تانينات، غلوسيدات، قلاويدات، كريما، حروق، ندوب.

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General Introduction

Medicinal plants have fascinated humanity for centuries, offering inherent healing properties and forming an integral part of traditional medicine across cultures. The use of medicinal plants in Algeria is deeply rooted in the culture and tradition of the country. They are used to treat various ailments and are considered a natural alternative to conventional medications .

The exploitation of these botanical treasures in the pharmaceutical field presents several advantages. Plants provide an abundant source of natural compounds, often with fewer undesirable side effects compared to synthetic drugs. Furthermore, medicinal plants can offer a diversity of active compounds, enabling the exploration of a wide range of potential therapeutic pathways.

Medicinal plants include a diversity of plant species, each possessing unique bioactive compounds and specific therapeutic potentials. They exhibit specific pharmacological actions ranging from pain relief and inflammation reduction to their ability to combat microbial infections and promote overall well-being.

Our work aims to evaluate the antimicrobial activity of four medicinal plants: *Artemisia herba alba, Brocchia cenirea, Moringa oleifera,* and *Aloe vera.*

In a context where an increasing number of consumers are concerned about their health, well-being, and the environmental impact of the products they use, organic healing creams are meeting this growing demand. The development of these creams is of significant commercial importance due to their unique advantages over conventional products. They are formulated using natural and environmentally friendly ingredients.

Therefore, our second objective is to develop a galenic formulation in the form of a cream, specifically an organic healing cream based on one of these plants, namely aloe vera. The goal is to assess its healing effects and the vital role this plant plays in wound healing and burn treatment.

This work is structured into three chapters:

Firstly, a literature review where the subject terms and key principles will be defined, including the characteristics of these four medicinal plants and the bacterial and fungal strains used in determining the antimicrobial effect in Chapter 1.

Secondly, Chapter 2 will cover the materials and methods used, and Chapter 3 will present the obtained results and their discussion.

Lastly, a general conclusion will summarize the main findings and provide future perspectives

Chapter 1
Bibliographic synthesis

1.1. Generality on medicinal plants:

1.1.1. Introduction:

Throughout history, humans have depended on Nature to fulfill their fundamental needs, including the production of food, shelters, clothing, transportation, fertilizers, flavors, fragrances, and, importantly, medicines. Traditional medicine systems, rooted in plants, have been developed and utilized for thousands of years, continuously offering humanity new remedies. While some of the attributed therapeutic properties of plants have been found to be incorrect, medicinal plant therapy is grounded in the empirical knowledge accumulated over countless centuries. The earliest recorded evidence of such practices, inscribed on clay tablets using cuneiform script, originates from Mesopotamia around 2600 BC. Among the substances employed were oils derived from Cedrus species (Cedar) and Cupressus sempervirens (Cypress), Glycyrrhiza glabra (Licorice), Commiphora species (Myrrh), and Papaver somniferum (Poppy juice). Remarkably, these substances are still employed today for treating a wide range of ailments, spanning from coughs, colds, and parasitic infections to inflammation ***(Gurib-Fakim, A. 2006).***

1.1.2. History:

Pinpointing the precise timeline of plant usage as medicine poses a significant challenge. However, evidence suggests that the cultivation of plants for medicinal purposes dates back approximately 60,000 years ***(Solecki R, Shanidar IV,1975)***. *Ancient* scripts documenting the knowledge of medicinal plants can be traced back to nearly 5,000 years ago in regions such as India, China, and Egypt. Similarly, in Greece and Central Asia, such scripts have been found dating back at least 2,500 years ***(Ang-Lee MK, Moss J, Yuan CS,2001)***. Since ancient times, humans have instinctively turned to nature in their quest to cure illnesses. While the initial utilization of animals for medicinal purposes may have been instinctive, this innate approach was also extended to the use of plants ***(Stojanoski N,1999)***. During that era, knowledge about the causes of diseases, beneficial plants for treatment, and methods of their utilization was limited. As a result, the approach to medicinal plants was entirely empirical. However, over time, the reasons behind using specific medicinal plants to treat certain ailments began to be unraveled. Consequently, the utilization of medicinal plants transitioned from an empirical PREPARATION_of medicinal drugs using plants can be traced back to a Sumerian clay slab discovered in Nagpur, dating back almost 5000 years ago **(*Qiu J.2007)*** According to inscriptions and historical records, the ancient Egyptians and Chinese were among the earliest human civilizations known to have utilized plants as medicine. Their use of medicinal plants for healing purposes can be traced back more than 27 centuries before the Common Era (BC). These civilizations played a significant role in the early development and advancement of herbal medicine practices ***(Schippmann UW, Leaman D, Cunningham AB,2006).***The ancient Greeks possessed knowledge about the healing properties of various medicinal plants. Figures such as Hippocrates, the founder of Greek medicine, and Aristotle, who was a student of Hippocrates, utilized medicinal plants for treating diseases. Subsequently, Theophrastus, a Greek scientist, established the School of Medicinal Plants. Furthermore, Pedanius Dioscorides, a physician and surgeon who lived between 75-45 BC, compiled an encyclopedia titled De Materia Medica. This comprehensive work consisted of scientific studies on 600 therapeutic medicinal plants, providing a detailed account of their properties ***(Lindberg Madsen H, Bertelsen G,1995/ Zargari A.1992).***

1.1.3. Definition:

The World Health Organization (WHO) provided a precise definition of medicinal plants:

a medicinal plant refers to any plant that possesses substances within its organs that can be utilized for therapeutic purposes or serve as precursors for semi-synthetic pharmaceuticals. This definition allows for a clear distinction between two categories:

*Medicinal plants that have undergone scientific research and have established therapeutic properties or are recognized as precursors for specific molecules.

*Plants used in traditional medicine that are considered medicinal, but their therapeutic properties have not been extensively studied through scientific investigation.

In summary, this definition enables the differentiation between scientifically validated medicinal plants and those used in traditional medicine that require further scientific exploration ***(Penso, G. 1980).***

1.1.4. Plants in modern medicine:

As per the World Health Organization (WHO), a significant majority, over 80%, of the global population primarily depends on traditional medicines, predominantly derived from plants, as their primary form of healthcare ***(Farnsworth nr,1985)***. This percentage encompasses not only the vast populations of countries like China, India, and other developing nations but also includes numerous advanced countries around the world ***(Ganesan a,2008)***. Despite the increasing prominence of synthetic medicines, which have been specifically developed in laboratories and proven effective in treating diseases, it is crucial to acknowledge that certain medications can have adverse effects on the body. As a result, the significance of medicinal plants and their derived products is gaining recognition, and public confidence in their use is steadily growing. This recognition stems from the understanding that medicinal plants offer alternative remedies with potentially fewer detrimental consequences ***(zergari a,1992)***. At present, clinical, pharmaceutical, and chemical research on traditional drugs, primarily sourced from plants, forms the foundation of numerous early drugs. Examples include Aspirin (derived from willow bark), Digoxin (from Foxglove), Morphine (obtained from Opium poppy), Quinine (extracted from Cinchona bark), and Pilocarpine (derived from Maranham Jaborandi). It is estimated that over 50% of the existing drugs in use today are derived, directly or indirectly, from medicinal plants. This highlights the continued significance of medicinal plants in the development and production of pharmaceutical treatments. ***(yernell E, abscal k,2002/hervey al,2008).*** Phytotherapy, the use of plant-based remedies for therapeutic purposes, is experiencing a significant and continuously expanding global adoption. As a result, there is a notable shift in the global trend towards herbal drugs, which can be seen as a return to nature for the prevention and alleviation of diseases and discomfort. Nature has long served as the abundant source of medicinal herbs, providing a rich repertoire of natural remedies for human well-being ***(fabrieant DS,fransworth Nr,2001).***

1.1.5. Secondary metabolites of plants:

Secondary metabolites can be categorized based on different criteria, such as their chemical structure (e.g., presence of rings or sugars), composition (e.g., containing nitrogen), solubility in solvents, or biosynthetic pathways (e.g., phenylpropanoid for tannin production). Generally, secondary metabolites are classified according to the pathway by which they are synthesized ***(Harborne& all,1996).*** Phenolics, Terpenes and Steroids, and Alkaloids and Flavonoids are three major families of large molecules that are generally recognized ***(bourgaud & all,2001).***

1.1.5.1. Phenolic:

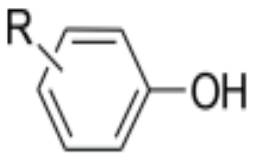
Phenolics are likely the largest group of plant secondary metabolites, sharing the presence of one or more phenol groups as a common characteristic. They can range from simple structures with one aromatic ring to highly complex polymeric substances and are found in many plants where they contribute significantly to the color, taste, and flavor of various herbs, foods, and drinks. Some phenolics, such as quercetin, are valued for their anti-inflammatory activities, while others like silybin have antihepatotoxic properties. Genistein and daidzein exhibit Phyto-estrogenic activity, and naringenin has insecticidal properties. Additionally, many phenolic molecules, particularly flavonoids, are potent antioxidants and free radical scavengers ***(Hussein, R. A., & El-Anssary, A. A,2019).***

a) Classification:

According to Al Mamari (2021), phenolic compounds can be classified broadly into simple and polyphenolic compounds.

- **Simple phenolic compounds:**

Simple phenolic compounds are characterized by the presence of one phenol unit or a derivative of it and are essentially substituted phenol compounds. They are typically represented by a C6 general skeleton structure **(*Al Mamari, H. H. (2021)).***



General structure of simple substituted phenols (*Al Mamari, H. H. (2021)).*

Simple substituted phenolic compounds can fall into two categories: hydroxyphenols or dihydroxy benzenes. Examples of these compounds include

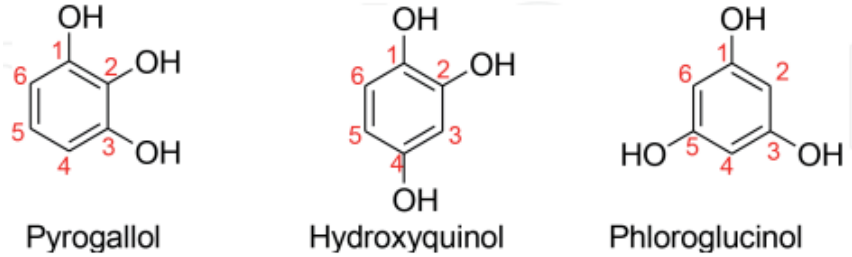
- catechol (1,2-dihydroxybenzene)
- resorcinol (1,3-dihydroxybenzene)
- hydroquinone (1,4-dihydroxybenzene)



The structure of hydroxyl-substituted phenols ***(Al Mamari, H. H. (2021)).***

Simple substituted phenolic compounds can also include dihydroxyphenols or trihydroxybenzenes, such as :

- pyrogallol (1,2,3-trihydroxybenzene)
- hydroxyquinol (1,2,4-trihydroxybenzene)
- phloroglucinol (1,3,5-trihydroxybenzene)



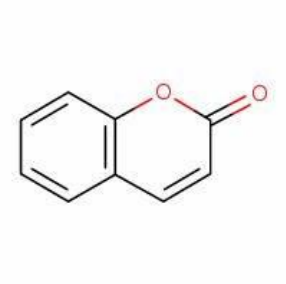
The structure of dihydroxyl-substituted phenols ***(Al Mamari, H. H. (2021)).***

- **Phenolic acids:**

Phenolic compounds that contain a carboxylic acid group are known as phenolic acids. If the carboxylic acid group is directly bonded to the phenol ring, the compound is referred to as a hydroxybenzoic acid. On the other hand, if the carboxylic acid group and the phenol ring are separated by two doubly bonded carbons (a C=C bond), the compounds are called hydroxycinnamic acids ***(Al Mamari, H. H. (2021)).***

- **Coumarins:**

Coumarins are a class of secondary metabolites that are commonly found in higher plants and some microorganisms and animals. These compounds are 1,2-benzopyrones and consist of a benzene ring linked to a pyrone ring. Coumarins are synthesized via the general phenylpropanoid pathway and the structural core of coumarins is 2H-1-benzopyran-2-one or "basic coumarin". Coumarins can be classified into two categories: complex and simple coumarins, based on modifications to this core structure. Coumarins that are more complex in structure are formed by adding heterocyclic compounds onto the basic coumarin core. These complex coumarins can be further categorized into different subtypes, including furanocoumarins, pyranocoumarins, phenylcoumarins, dihydrofurocoumarins, and biscoumarins ***(Medina & al. 2015).***



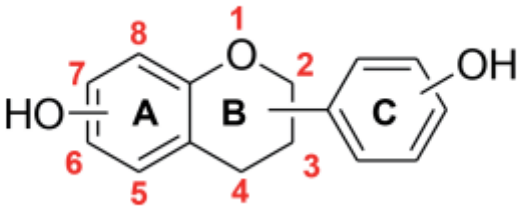
Basic coumarins (*Al Mamari, H. H. (2021).*

- **Polyphenols:**

Polyphenolic compounds are characterized by the presence of more than one phenol unit and are commonly referred to as "polyphenols." These compounds have a C15 general skeleton representation **(*Al Mamari, H. H. (2021).***

- **Flavonoids:**

Flavonoids are natural pigments found in plants and are derived from benzo-g-pyrone, which is also known as chromone ***(Havsteen, B. H. (2002).*** They are formed in plants from the amino acids phenylalanine and tyrosine, as well as malonate. The structure of flavonoids is based on the flavan nucleus, which consists of three rings labeled A, B, and C and a total of 15 carbon atoms arranged in a C6-C3-C6 configuration **(Xin & al., 2014).**



General structure of flavonoids *(Al Mamari, H. H. (2021)).*

- **Tannins*:***

Tannins are phenolic compounds with high molecular weight, typically ranging from 500 Da to over 3000 Da. They are commonly found in various parts of plants, including leaves, bark, fruit, wood, and roots, mainly in vacuoles. Tannins are known to play a crucial role in the plant's defense mechanism against herbivores, birds, and insects ***(Hagerman & Buther, 1981/ Hassanpour & al, 2011(a)).*** Although some tannins have a higher molecular weight, they are generally soluble in water at temperatures between 20-35°C. These compounds can form complexes with proteins, starch, cellulose, and minerals, thanks to the presence of multiple structure units with free phenolic groups. Tannins are present in both flowering and non-flowering plants, including species like Acacia spp, Sericea lespedeza, and Lotus spp, which are commonly used as pasture ***(Hassanpour & al, 2011(a)).***

1.1.5.2.Terpenes:

Terpenes are a large and diverse group of over 30,000 lipid-soluble compounds that contain one or more 5-carbon isoprene units. Terpenoids, which are classified based on the number of isoprene units they contain, are derived from isoprene itself, a compound that is synthesized and released by plants. Hemiterpenes, which contain a single isoprene unit, monoterpenes (2 units), sesquiterpenes (3 units), diterpenes (4 units), sesterpenes (5 units), triterpenes (6 units), and tetraterpenes (8 units) are examples of terpenoids **(*Kennedy, D. O., & Wightman, E. L. (2011)).*** Myrcene, beta-caryophyllene, and limonene are among the known antipsychotic terpenoids. However, these compounds also have anti-depressant properties, as they can activate and suppress the cannabinoid receptor two ***(Ajao & al,2018).***

1.1.5.3. Alkaloids:

The term "alkaloids" is derived from "alkaline" and was used to describe any nitrogen-containing base. Alkaloids are typically organic bases that form salts with acids and produce alkaline solutions when dissolved. Flowering plants are a major source of alkaloids ***(Roy, A,2017).*** Alkaloids are a category of chemical compounds found in nature that are primarily characterized by the presence of basic nitrogen atoms. Although some compounds that are related to alkaloids possess neutral or weakly acidic properties, they are still included in this group. Synthetic compounds with similar structures are also sometimes referred to as alkaloids. Along with carbon, hydrogen, and nitrogen, alkaloids may contain other elements such as oxygen, sulfur, and, less commonly, chlorine, bromine, and phosphorus ***(Babbar, N,2015).***

1.1.5.4.Saponins:

Saponins get their name from the Latin word "sapo" which means soap, as they exhibit surfactant properties and create a stable foam similar to soap when shaken in water. Saponin is a chemical term that refers to a collection of high molecular weight glycosides. These glycosides consist of a glycan moiety linked to an aglycon, also known as a genin or sapogenin ***(Hostettmann & Marston 2005).***Saponins are classified into two categories based on the chemical structure of their aglycon or genin component. Triterpenoid saponins consist of 30 carbon atoms and are predominantly found in the class of Magnoliopsida, while steroidal saponins have 27 carbon atoms and feature either a 6-ring spirostane or a 5-ring furostane skeleton. Steroidal saponins are nearly exclusively present in the class of Liliopsida ***(Sparg & al. 2004).***

1.2.Plants Studied:

1.2.1.*Moringa oleifera* :

1.2.1.1. Definition:

M. Oleifera is commonly referred to as either Horse Radish tree (referring to the taste of its roots) or Drumstick tree (describing the shape of its pods) ***(Shih & al., 2011)*** while less frequently referred to as 'The Tree of Life' or 'Miracle Tree' due to its economical importance and versatility ***(Abe & Ohtani 2013/ Bakre & al., 2013)***. A single genus with 14 known species, M. oleifera is the most widely known and utilized of these ***(Morton, 1991).*** *Moringa oleifera* is referred to as “Moringa”, it is considered one of the world’s most useful trees. Almost every

part of moringa tree can be used for food or other beneficial applications ***(Quattrocchi & Umberto, 2000).***

1.2.1.2.Botanic description:

Moringa oleifera, also known as the Tree of Life or Miracle Tree, is a deciduous tree with sparse foliage. From a distance, it can resemble a leguminous species, but is easily recognizable by its fruit. The tree can reach a height of 8 meters and a diameter at breast height of 60 centimeters. Its bark is smooth and dark gray, and the twigs and shoots are densely hairy. The tree has a wide, open crown that is typically umbrella-shaped and deep-rooted. The wood of Moringa oleifera is soft according to Orwa et al. (2009*).**(Taher & al,2017) .***

- **Leaves:**

The tree bears large, alternate leaves that grow on petioles measuring 4-15 cm long. These tri-pinnate leaves range in size from 6.5 cm to 90 cm in length, with most falling between 25-60 cm long. The leaves have pulvinate main branches numbering 5-11***(Taher & al,2017)***.



Figure 1.1: Moringa Leaves **(https://sc04.alicdn.com)**

- **Fruits:**

The fruit of the Moringa tree has an elongated shape, similar to that of beans or pea pods. It is initially white during the vegetative stage, but matures into a brown color. Each fruit typically contains 5 to 20 seeds, which are highly valued by locals. The fruit has a taste similar to asparagus and its appearance has led to the tree being referred to as the "drumstick tree***"(Taher & al,2017)***



Figure 1.2 : Moringa fruits **(https://i.pinimg.com)**

- **Flowers:**

Moringa oleifera produces fragrant flowers that are typically creamy white and have yellow stamens. The flowers are around one inch in diameter and begin to bloom at eight months old, with subsequent blooming occurring yearly from April to September ***(Taher & al,2017)***.



Figure 1.3: Moringa flowers **(https://lh5.ggpht.com**)

- **Seeds:**

Moringa seeds come from the pods of the Moringa oleifera tree, also known as the drumstick tree. When fresh and unripe, the seeds are soft, but they become hard and bean-like when dried. The seeds have a grayish-white color and are characterized by distinctive wing-like structures. They can be prepared by steaming, boiling, or roasting for various *uses* ***(Taher & al,2017)***.



Figure 1.4: Moringa seeds **(https://media.istockphoto.com)**

- **Other parts of *M. oleiferea*:**

The tree typically has a broad, umbrella-shaped crown with a single main trunk and a large underground rootstock. Its trunk, which is usually 10-45 cm wide, is covered in a pale-grey bark, although it may sometimes reach up to 60 cm in diameter. The branches of the tree are borne on stalks that are 1-3 cm long and each branch has 5-11 smaller branches **(*Taher & al,2017).***

1.2.1.3.Taxonomic description:

Table 1.1: classification of Moringa plant ***(Taher & al,2017)***.

Classification of plant	
Kingdom	*Plantae*
Order	*Brassicales*
Family	*Moringaceae*
Genus	*Moringa*
Species	*Moringa oleifera*
Synonyms	*Guilandina moringa L.*

1.2.1.4.Distribution:

Moringa oleifera, which belongs to the monogeneric family Moringaceae, is the most commonly cultivated species of the genus. The plant is indigenous to the sub-Himalayan regions of India, Pakistan, Bangladesh, and Afghanistan. It is a fast-growing tree with several names such as horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna, or Ben oil tree. Moringa has a rich history of use, dating back to the ancient Egyptians, Greeks, and Romans. It is now widely cultivated and naturalized in tropical regions around the world. The tree has a low-quality timber and is primarily grown for traditional medicinal and industrial purposes. Moringa is already an important crop in India, Ethiopia, the Philippines, and Sudan, and is being grown in several other regions such as West, East, and South Africa, tropical Asia, Latin America, the Caribbean, Florida, and the Pacific Islands. The plant's leaves, seeds, flowers, and pods are all edible and have been consumed by humans for centuries ***(Fahey, J. W. (2005)).***



Figure 1.5: Distribution of Moringa in world **(Anzano& al,2022)**

1.2.1.5.Chemical composition:

Moringa oleifera is abundant in compounds that contain the simple sugar, rhamnose, as well as a distinct group of compounds called glucosinolates and isothiocyanates **(Fahey et al., 2001; Bennett et al., 2003)**. Two alkaloids, moringine and moringinine, have been identified in the stem bark **(Kerharo, 1969)**. The stem of M. oleifera has been found to contain vanillin, β-sitosterol, β-sitostenone, 4-hydroxymellin, and octacosanoic acid **(Faizi et al., 1994)**. The purified, whole-gum exudate of M. oleifera has been found to contain L-arabinose, -galactose, -glucuronic acid, and L-rhamnose, -mannose, and -xylose, while mild hydrolysis of the whole gum with acid yielded a homogeneous, degraded-gum polysaccharide consisting of L-galactose, -glucuronic acid, and L-mannose **(Bhattacharya et al., 1982)**. The flowers contain nine amino acids, sucrose, D-glucose, traces of alkaloids, wax, quercetin, and kaempferat. They also contain flavonoid pigments such as alkaloids, kaempherol, rhamnetin, isoquercitrin, and kaempferitrin **(Faizi et al., 1994; Siddhuraju and Becker, 2003).** Antihypertensive compounds, such as thiocarbamate and isothiocyanate glycosides, have been isolated from the acetate phase of the ethanol extract of Moringa pods **(Faizi et al., 1998)**. The fruit has been shown to contain cytokinins**(Nagar et al., 1982).** A novel compound, O-ethyl-4-(α-L-rhamnosyloxy) benzyl carbamate**,** and seven other known bioactive compounds, 4(α-L-rhamnosyloxy)-benzyl isothiocyanate , niazimicin**,** 3-O-(6′-O-oleoyl-β-D-glucopyranosyl)-β-sitosterol , β-sitosterol-3-O-β-D-glucopyranoside , niazirin, β-sitosterol , and glycerol-1-(9-octadecanoate) , have been isolated from the ethanol extract of the Moringa seed **(Guevara et al., 1999).**

I.2.1.6.Medicinal uses:

Moringa is believed to possess numerous medicinal properties, as its various parts have been used alone or in combination with other plants to treat a wide range of illnesses and diseases ***(Fuglie, 1999/ Pamok & al., 2011)***The leaves of Moringa are commonly used in traditional medicine to treat various ailments such as malaria, typhoid fever, parasitic diseases, arthritis, skin diseases, swelling, hypertension, diabetes, and genito-urinary ailments**(*Leone & al., 2015)***. Additionally, Moringa leaves are used to stimulate lactation and enhance the immune system, particularly for managing symptoms related to HIV/AIDS **(*Anwar & al., 2007; Popoola & Obembe, 2013; Sivasankari & al., 2014),*** it's can also act as cardiac stimulants and contraceptive remedies. To treat these ailments, they can be consumed either in their raw or dried form, or in the form of an aqueous infusion extract ***(Leone & al., 2015).*** Local people in Nigeria have reported using the leaves of the Moringa species to treat HIV infections and cancer **(*Popoola & Obembe, 2013).***Due to insufficient scientific information, there are limited published studies on the specific uses of Moringa for treating HIV infections and cancer. However, the bark of the tree can be boiled in water or soaked in alcohol to extract decoction and extract, respectively. These extracts have been traditionally used to treat stomach ailments (such as stomach pain, ulcers, and aiding digestion), poor vision, joint pain, diabetes, anemia, and hypertension***(*Abe & Ohtani, 2013/ Popoola & Obembe, 2013*),**toothache, hemorrhoids, uterine disorder **(*Popoola & Obembe, 2013/ Yabesh & al., 2014).*** In a wellknown practice to local people in Nigeria, Moringa seeds are used to sediment impurities of water ***(Popoola & Obembe, 2013).*** The roots can be soaked in water or alcohol and boiled to obtain a decoction or extract that is used as a remedy for toothache, as well as an anthelmintic and antiparalytic agent ***(Anwar & al., 2007/Popoola & Obembe, 2013/ Sivasankari & al., 2014)*** they used as remedies for toothache and as anthelmintic and antiparalytic drugs, as well as sex stimulants.

Additionally, the flowers are utilized to produce aphrodisiac substances and to treat inflammations, muscle diseases, hysteria, tumors, and enlargement of the spleen ***(Anwar & al., 2007/ Yabesh & al., 2014).*** Several screening tests and ethnopharmacology studies have confirmed the activities of Moringa against the aforementioned diseases ***(Pamok & al., 2011; Abe & Ohtani, 2013; Popoola and Obembe, 2013/ Sivasankari & al., 2014/ Yabesh & al., 2014).***

1.2.2. *Brocchia cinerea:*

1.2.2.1. Definition:

Brocchia cinerea, also known as Cotula cinerea, is a plant species belonging to the Asteraceae family, which is recognized for its abundance in essential oils. This plant has been traditionally utilized for the treatment of various bacterial infections and diseases ***(Idm'Hand & all E,2020/Lakhdar, M. 2018/ Telli, A & all ,2016)*** due to its good preservative properties, additionally, it is used to filter goat butter **(*Bouziane, M2013)***. *B. cinerea* has other biological activities: the powder of this species has an effect on the mycelial growth of *Penicillium italicum* (43.08%), the agent responsible for the blue mold of citrus fruits **(*Agour, A & all 2022).***

1.2.2.2. Botanic description:

Brocchia cinerea is a small herbaceous plant covered with a woolly white coating ***(M. Djellouli& all 2013/Bensizerara & al., 2012)*** It is an annual plant that grows to a height of 5-15 cm, and its stems are either erect or diffuse, with thick leaves that are divided into 3-5 obtuse teeth in the upper part. The stems can range from 10-40 cm in length and the capitulum, which is woolly and has tubular flowers, is typically 6-10 mm in diameter. The plant also has brown buds that turn golden yellow when fully mature ***(m. djellouli & all 2013 / i. talibi, n. & all 2011/ h. dendougui & all 2012/m. markouk & all 1999 / p. ozenda 1983)***



Figure 1.6 : *Brocchia cinerea* plant **(https://live.staticflickr.com)**

1.2.2.3. Taxonomy:

Table 1.2: Classification of *Brocchia cinerea* **(*Lakhdar, M. 2018).***

Brocchia	Classification
Kingdom	*Plantae*
Subkingdom	*Tracheobionta*
Family	*Compositae and the Cichorioideae*
subfamily	*Asteroideae*
Tribe	*Anthemideae,*
subtribe	*Cotulineae*
Division	*Magnoliophyta*
Superdivision	*Spermatophyta*
Class	*Magnoliopsida*
Sub-class	*Asteridae*
Order	*Asterales*
Genus	*Cotula*
Species	*Cinerea*
Binomial name	*Cotula cinerea Del*
Synonym	*Brocchia cinerea Del*
Vernacular names	*Gartoufa, Gartoufa beida, Chouihiya, Chihia, Shihit El Ebel, Chiria, Robita*

1.2.2.4.Distribution:

- ***In the world generally:***

B. cinerea is typically found in wadi beds with gravelly sandy soils that are non-saline, and it has a preference for sand-loamy soils ***(Boulos, L. 1983)***. Its distribution is widespread in North Africa, particularly in Saharan regions of Algeria and Morocco, the Red Sea region, the Sinai, the Qattara Depression, and Mali ***(Ozenda P1991).***

- ***In Algeria:***

Brocchia cinerea, also known as Cotula cinerea, is a type of xerophytic plant and one of three species in the Cotula genus that can be found in Algeria. ***(Dendougui & al., 2012/ Maiza & al., 1993)*** It belongs to the Asteraceae family and is commonly found in the Sahara Desert and sandy regions ***(Quezel & Santa, 1963).*** One of the regions where it is found is Oued Souf ***(Halis, 2007/Ozenda, 1977)***. where it is locally known as "Shihia/Shihit El Ebel"***(Halis, 2007).***

1.2.2.5. Chemical constituent of oil:

- **In Ouargla (South-eastern Algeria):**

Table 1.3: Chemical compositions of *Brocchia cinerea* oil in *Ouargla* ***(Bouziane & all,2013)***

Chemical compositions	Percentage
Camphor	(10.54 %);
Thujone	(47.72 %);
Camphor	(10.54 %);
Eucalyptol	(6.37 %);
Acetate	(4.17 %),
Terpinen-4-ol	(2.77 %).
Santolinatriene	(8.00%);

- **In Bachar (Southwest Algeria):**

Table 1.4: Chemical compositions of *Brocchia cinerea* oil in Bachar ***(Djellouli M& all,2015)***

Chemical compositions	Percentage
(E)-citral	(24.01%)
cislimonene epoxide	(18.26%)
thymol methylether	(15.04%)
carvacrol	(15.03%)
trans-carveol	(13.79%)
carvone	(3.06%)
transpiperitol	(2.54%)

1.2.2.6. Medicinal uses:

Cotula cinerea has been used extensively in traditional medicine to treat various ailments such as colic, cough, diarrhea, and digestive disorders. The plant is often administered through decoction, maceration, infusion, or inhalation *methods* ***(Bellakhdar J,1997 / Djellouli M & all ,2013)***. Moreover, it is valued in traditional medicine for its antiseptic, antipyretic, analgesic, and anti-inflammatory properties and is also employed to treat rheumatism ***(Beloued A,2005 / Hammiche V&all 2006)*** Is also use for tea additive to enhance the taste of tea. It is also valued for its preservative properties and used to filter goat butter ***(Bouziane M &all 2013)***

1.2.3. *Aloe vera:*

1.2.3.1. Definition:

Aloe vera is a type of succulent plant, meaning it is a xerophyte adapted to surviving in regions with limited water availability. These plants are known for possessing ample water storage tissue ***(Hamman JH,2008)***. *Aloe vera L*. (Liliaceae) is a semi-tropical plant that has long been utilized by herbalists to treat various human ailments. It is closely related to other members of the Lily family, including onion and garlic ***(Mccouley, 1990/ Shellon,1996/Davis & Robson, 1999)***

One of the primary characteristics of the Aloe vera plant is its exceptionally high-water content, which ranges from 99-99.5%. The remaining 0.5-1.0% solid material contains more than 75 potentially active compounds, including vitamins that are soluble in water and fat, minerals, enzymes, complex and simple polysaccharides, phenolic compounds, and organic acids. Aloe vera is also a rich source of 19 out of the 20 essential amino acids required by our body, which help in the smooth functioning of our complex enzyme system ***(Hamman JH ,2008)***

1.2.3.2. Definition of Aloe vera gel:

Aloe vera gel is an aqueous extract that is obtained from the mucilaginous leaf pulp of Aloe barbadensis Miller. It contains more than 70 biologically active compounds and is believed to possess several properties **(*Grindlay D & all 1986)*** including anti-inflammatory, anti-oxidant, immune-boosting, anti-cancer, healing, anti-aging, and anti-diabetic properties. While there is limited scientific evidence to support these claims, a randomized trial has demonstrated that topical application of aloe vera gel is more effective than a placebo in treating plaque psoriasis ***(Langmead L,& all ,2002).***



Figure 1.7: Aloe vera gel **(https://www.aloevitasante.com*)***

1.2.3.3.Botanic description:

Aloe vera is a perennial, xerophytic, succulent plant with turgid green leaves. It is a stemless or very short stemmed plant growing up to 60 to 100 cm tall, spreading by offsets.



Figure 1.8: Aloe vera plant **(https://www.leaderplant.com**)

- **Leaves:**

The leaves of aloe vera plant are elongated pointed and joined in rosette pattern / also the leaf resembles a long triangular sheaf with two external membranes which are green and leathery. Inside this tough resistant covering is the gel, which presents itself as a compact, gelatinous mass with a translucent pearly *aspect* ***(Ramachandran & all 2012, Vogler BK Ernst E (1999), Poor MR 2002).***



Figure 1.9 : The leaves of aloe vera plant **(https://cdn.jurassicfruit.com)**

- **Flowers:**

The flowers of A. vera comes out in summer on a spike up to 90 cm tall, each flower being pendulous, with a yellow tubular corolla and 2 to 3 cm long.



Figure 1.10 : The Fowers of aloe vera plant **(https://worldoffloweringplants.com)**

1.2.3.4.Taxonomy:

Table 1.5: The taxonomy of aloe vera ***(Own elaboration based on Albiter, 2010)***

Kingdom	Plantae
Division	Magnoliophyte
Class	Liliopsida
Order	Liliales
Family	liliaceae
Subfamily	asphodeloideae
Gender	aloe
Species	Aloe vera

1.2.3.5. Distribution:

Aloe vera is distributed throughout most of Africa, Southern Arabia, and Madagascar, excluding regions with rainforests or arid deserts. Some species have been cultivated and transported to the Mediterranean region and beyond, including Japan in the east and America in the west ***(Grindlay, D & all 1986).***

1.2.3.6. Chemical Composition:

Aloe Vera Gel is composed primarily of water, comprising approximately 99.3% of its content. The remaining 0.7% is made up of solids, consisting mainly of glucose and mannose polysaccharides. Aloe Vera Gel is also rich in enzymes, with a content of approximately 92 enzymes. This high enzyme content makes it a valuable resource for aiding in the absorption of nutrients and purifying the body. Studies have identified 75 different ingredients in the Aloe Vera leaf, each of which possesses various medical benefits. These ingredients can be divided into several categories ***(Davis RH & ALL, 2006).***

1.2.3.7. Medicinal uses:

- **The use of aloe vera gel:**

In Trinidad and Tobago, Aloe vera gel is used as a traditional remedy for hypertension. Its most common use in traditional medicine is for treating burn wounds, as it aids in the healing process, reduces inflammation, and prevents tissue scarring. Historical records show that the gel was first described by Dioscorides, who used it to treat wounds and mouth infections, relieve itching, and heal sores. Lans CA 2006The use of aloe vera gel as a household remedy in the United States was triggered by reports of its beneficial effect on radiation dermatitis52 followed by a boom in cultivation in the 1930**(Collins CE, &Collins c,1935).**

- **Primary Uses**(determined by clinical trials):

  - ✓ **External:**
    - Mild to moderate burns **(*Thamlikitkul V & all,1951/ Akhtar MA, Hatwar SK & all / erythema Reuter J & all,2008)***
    - Genital herpes ***(Syed TA, Afzal M, Ashfaq AS, 1997/ Syed TA& all,1996)***
    - Seborrheic dermatitis ***(Vardy AD& all,1999)***

  - ✓ **Internal:**

    - Adjunct therapy of spontaneous fibro sarcomas in dogs and cats**. *(Dal'Belo SE, Gaspar LR, Maia Campos PM & all,2006/Harris C & all,1991)***
    - Psoriasis vulgaris *(**Syed TA & all,1996)***
    - *Skin moisturizer* ***(King GK & all,1995)***
    - Type 2 diabetes ***(Tanaka M*& all,2006 */Agarwal OP ,1985)***
    - Malignancies and immunodeficiency viruses in cats ***(Yates KM & all,1992)***
    - Oral lichen planus infections (**Choonhakarn C*& all,2008*/Hayes SM & all,1999)**
    - Angina pectoris **(*Agarwal OP& all,1985)***
    - Ulcerative colitis **(*Langmead L*& all,2004 */ Avijgan M. & all,2004)***
    - UV-induced erythema14 Kidney stones ***(Kirdpon S & all,2006 /Kirdpon S & all,2006)***
    - Alveolar osteitis ***(Poor MR, Hall JE, Poor AS & all,2002)***

1.2.4. *Artemisia herba-alba:*

1.2.4.1. Definition:

Artemisia herba-alba, commonly known as "desert wormwood" in English, "armoise blanche" in French, and "shaih" in Arabic is a medicinal and aromatic dwarf shrub belonging ***(Hudaib & Aburjai, 2006 /Dahmani-Hamzaoui& Baaliouamer,2010/ Tilaoui &al., 2011),*** that grows wild in arid areas it is commonly known as Aster or Compositae family, comprising about 1535 genera and 23,000 species.one of the most economically important and widespread of this family genus, includes 400 species ***(Judd & al., 2002)*** For centuries, various cultures have employed the above-ground portions of A. herba-alba in traditional medicine. The plant is commonly utilized to treat a range of ailments, including inflammation-related disorders (such as colds, coughs, bronchitis, and diarrhea), infectious diseases (including skin ailments, scabies, and syphilis), as well as conditions like diabetes and neuralgias ***(Salido & al., 2004/Mohsen & Ali, 2009/ Mighri &al., 2010)***

1.2.4.2.Description Botanic:

Artemisia herba-alba is a greenish-silver perennial herb grows 20-40 cm in height it is a chamaeophyte (it means that the buds giving rise to new growth each year are borne close to the ground). The stems are rigid and erect. The grey leaves of sterile shoots are petiolate, ovate to orbicular in outline whereas leaves of flowering stems are much smaller. The flowering heads are sessile, oblong and tapering at base. The plants flower from September to December. Plants are oblong and tapering at base**.** ***(N. Feinbrun-Dothan (1978).****A. herba-alba* is an outcrossed species, and pollination is mostly anemogamous. Fruits are small achenes mainly dispersed by wind and water. The species is mostly diploid (2n = 2x = 18), and may be tetraploid (2n = 4x = 36) ***(Ferchichi, 1997).***



Figure 1.11 : *Artemisia herba-alba* plant **(https://www.floramaroccana.fr)**

1.2.4.3.Taxonomy:

Table 1.6: Taxonomy of *Artemesia herba alba* **(*L. Boulos (2002), Flora of Egypt, vol. III. Al-Hadara publishing, Cairo, Egypt)***

Artemesia herba alba	
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order:	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Tribe:	Anthemideae
Subtribe	Artemisiinae
Genus:	Artemisia L.,
Subgenus	Seriphidium
Species:	Artemisia herba-alba

1.2.4.4.Distribution:

Much attention has been given to the initial emergence of Artemisia, and evidence from fossilized pollen indicates that the genus originated in temperate Asia during the Late Eocene. ***(Miao & al., 2011)***. Artemisia species tend to thrive primarily in the Northern Hemisphere, with fewer species found in the Southern Hemisphere. This plant is cultivated in various regions including Asia, India, Central and Eastern Europe, temperate regions of America, Africa, and Australia, as well as in tropical areas ***(A.Septembre-Malaterre & all,2020).*** China has the highest number of Artemisia species, with 150 accessions, followed by the ex-USSR with 174 species, and Japan with approximately 50 reported species ***(Abad Martínez, M & all,2012)***.Propagation of the plant can be achieved through simple methods such as root division, root cuttings, or seed germination in the autumn. Wormwood is indigenous to temperate areas, and its habitats span from Europe, Scotland, and Scandinavia to the Mediterranean Sea, as well as regions as far south as North Africa and as far east as Siberia and Northern India. This plant has been introduced to North and South America, North and West Asia, New Zealand, and the Azores, where it has become naturalized ***(Judžentienė, A. 2016)***

➢ **In Algeria**

Artemisia is found in Algeria primarily in steppe areas along a 1200 km strip that stretches from the Tunisian border to the Moroccan border. This region is known for its nomadic sheep farming. The plant prefers dry and warm climates and often forms large populations in desert areas. It is most commonly found in highland regions but is not commonly found in thenorthern Sahara ***(BOUAFIA, A. (2022).***

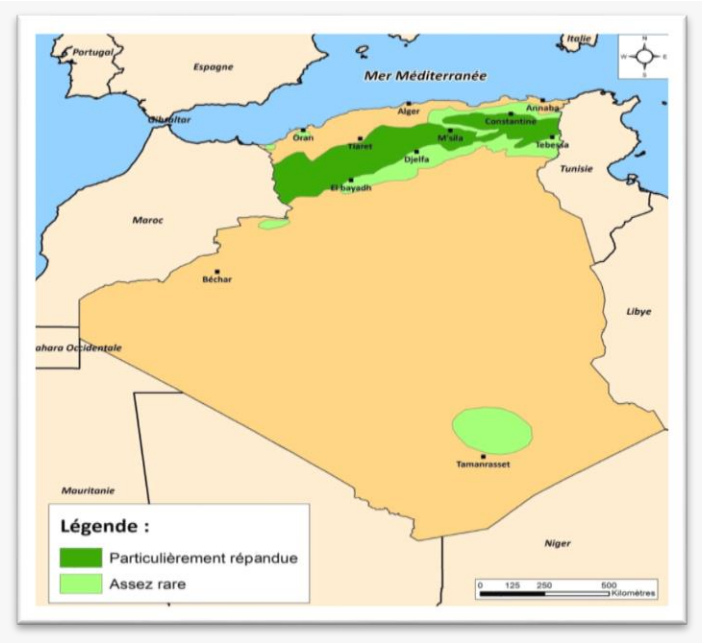


Figure 1.12: Geographical distribution of Artemisia herba-alba in Algeria ***(BOUAFIA, A. (2022).***

1.2.4.5.Chemical constituent:

➢ **Algeria Msila:**

The essential oil obtained by hydrodistillation from the aerial parts of *Artemisia herba-alba* Asso growing wild in M'sila-Algeria, was investigated using both capillary GC and GC/MS techniques. The oil yield was 1.02% based on dry weight. Sixty-eight components amounting to 94.7% of the oil were identifed, 33 of them being reported for the frst time in Algerian *A. herba-alba* oil and 21 of these components have not been previously reported in *A. herba-alba* oil.

Table 1.7: Chemical compositions of Artemisia herba-alba oil in Algeria msila ***(Dob, T & Benabdelkader, T. (2006))***

Chemical compositions in Algeria msila	Percentage
Camphor	19.4%
Trans-pinocarveol	16.9%
Chrysanthenone	15.8%
β-thujone	15%
Monoterpenoids	86.1%
Monoterpenes	3.1%

1.2.4.6.Usage medicinal:

A. herba-alba has been traditionally used in folk medicine to treat various diseases, it is used by many cultures since ancient times. It is used to treat inflammatory disorders (colds, coughing, bronchitis, diarrhea), infectious diseases (skin diseases, scabies, syphilis) and others (diabetes, neuralgias) ***(Abu-Darwish, M 2015)***.The essential oils derived from A. herba-alba have also been reported to possess antioxidant, antibacterial, antileishmanial, anthelmintic, antispasmodic, and anticholinesterase properties ***(Mighri & al., 2010/Abu-Darwish & al., 2015/Younsi & al., 2016).***

1.3. The bacterial strain:

1.3.1.*Escherichia coli:*

1.3.1.1. Definition:

E. coli belongs to the Enterobacteriaceae family of bacteria, and is the most common commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals. It is also one of the most significant pathogens associated with a variety of infections ***(Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004))***. E. coli is widely recognized as a normal inhabitant of the intestinal microflora of humans, other warm-blooded animals, and reptiles, and is considered a commensal organism that typically does not cause disease. However, E. coli is also one of the most common pathogens responsible for a range of diseases in both humans and animals. The unique characteristics of E. coli, including its ease of handling, complete genome sequence availability, and ability to grow under different conditions, have made it a valuable host organism in biotechnology. It is extensively used in various applications in both medical and industrial fields, and is the most commonly used microorganism in recombinant DNA technology ***(Yoon, S & all,2009)***.

1.3.1.2.Taxonomy:

Table 1.8: Taxonomy of Escherichia coli ***(Bergey's Manual of Systematics of Archaea and Bacteria)***

Domain	***Bacteria***
Phylum	*Proteobacteria*
class	*Gammaproteobacteria*
order	*Enterobacterales*
Family	*Enterobacteriaceae*
Genus	*Escherichia*
Species	*Escherichia coli*

1.3.1.3.Habitat:

Escherichia coli is a Gram-negative, nonspore-forming, facultative anaerobic bacterium found in the gut and feces of warm-blooded animals and reptiles ***(Berg, R.D. (1996), Gordon, D.M. & Cowling, A. (2003)).*** Escherichia coli is present in the gut microbiota, and Escherichia coli is the main aerobic organism in the gastrointestinal tract. Migrate in water and sediment. ***(Savageau, M.A. (1983)).*** Different E. coli hosts have different body sizes, gut morphologies, diets, digestion times, and microbiota. These properties can have a major impact on the

prevalence and density of E. coli, which varies from 0% to 100% and varies by more than 6 orders of magnitude between host species. In humans, the prevalence exceeds 90% **(*Penders, J & al 2006)*,** but only 56% in wild mammals, 23% in birds, and 10% in reptiles **(*Gordon, DM & Cowling, A,2003)*.** In the digestive tract, commensal E. coli strains are present in the large intestine, especially the cecum and large intestine. They are located in the mucus layer that covers the epithelium throughout the digestive tract and are released into the intestinal lumen along with degraded mucus components to be excreted in the feces **(*Poulsen, L.K. & al., 1994).***

1.3.1.4.Pathogenicity:

E. coli strains are classified into pathotypes (a group of strains of the same species that cause common disease) according to the type of virulence factors present and the clinical symptoms of the host: there are at least seven major pathogenic types of E. coli. while the three E.coli pathogenic types are extraenteric strains (ExPEC) ***(Kaper, J.B & al (2004).***

Table 1.9: Patogenicity of Escherichia coli ***(Bfp:* Bundle-forming pili/*LEE*: Locus for enterocyte effacement; *HUS*: haemolytic-uraemic syndrome/ *CFA*: colonization factor antigen/*AAF*: aggregative adherence fimbria/ *Daa*: diffuse adhesin/ *AIDA*: adhesin involved in diffuse adherence; *Ipa*: Invasion plasmid antigen *(Allocati, N & all,2013)***

Pathotype (acronym)	Diseases	Symptoms	Virulence factors
Enteric E.coli			
EnteroPathogenic E. coli (EPEC)	Diarrhoea in children	Watery diarrhoea and vomiting	Bfp, Intimin, LEE
EnteroHaemorrhagic E. coli (EHEC)	Haemorrhagic colitis, HUS	Bloody diarrhoea	Shiga toxins, Intimin, Bfp
EnteroToxigenic E. coli (ETEC)	Traveler's diarrhoea	Watery diarrhoea and vomiting	Heat-labile and sheat-stable toxins, CFAs
EnteroAggregative E. coli (EAEC)	Diarrhoea in children	Diarrhoea with mucus and vomiting	AAFs, cytotoxins
Diffusely Adherent E. coli (DAEC)	Acute diarrhoea in children	Watery diarrhoea, recurring UTI	Daa, AIDA
EnteroInvasive E. coli (EIEC)	Shigellosis-like	Watery diarrhoea; dysentery	Shiga toxin, hemolysin, Cellular invasion, Ipa
Adherent Invasive E. coli (AIEC)	Associated with Crohn disease	Persistent intestinal inflammation	Type 1 fimbriae, Cellular invasion
Extraintestinal E. coli (ExPEC)			
UroPathogenic E. coli (UPEC)	Lower UTI and systemic infections	Cystitis, pyelonephritis	Type 1 and P fimbriae; AAFs, hemolysin
Neonatal Meningitis E. coli (NMEC)	Neonatal meningitis	Acute meningitis, sepsi	S fimbrie; K1 capsule
Avian Pathogenic E. coli (APEC)	Probable source of food-borne disease	-	Type 1 and P fimbriae; K1 capsule

1.3.2. Bacillus subtilis:

1.3.2.1.Definition

Bacillus subtilis is a remarkably diverse bacterial species that is capable of growth within many environments. Recent microarray-based comparative genomic analyses have revealed that members of this species also exhibit considerable genomic diversity. The identification of strain-specific genes might explain how B. subtilis has become so broadly adapted. The goal of identifying ecologically adaptive genes could soon be realized with the imminent release of several new B. subtilis genome sequences. As we embark upon this exciting new era of B. subtilis comparative genomics we review what is currently known about the ecology and evolution of this species **(*Earl, A. M., Losick, R., & Kolter, R,2008)***

1.3.2.2. Taxonomy:

Table 1.10: Taxonomy of Bacillus ***(Kovács, Á. T. 2019)***

Kindom	**Bacteria**
Phylum	Firmicuteus
Order	Bacillales
Family	Bacillaceae
Genus	Bacillus
Speiceis	Bacillus subtilus

1.3.2.3.Morphology:

The genus Bacillus comprises numerous rod-shaped Gram-positive bacteria that are motile via peritrichous flagella and are typically aerobic, although they may initially test positive in Gram staining during early stages of growth. These bacteria are highly diverse and have the unique ability to produce endospores that exhibit high resistance to adverse environmental conditions ***(Claus & Berkeley, 1986)***. The diversity of organisms within the genus is apparent through the broad spectrum of DNA base ratios, which range from approximately 32 to 69 mol% G + *C* ***(Claus & Berkeley, 1986)***, This range of DNA base ratios is notably wider than what is typically considered reasonable for a single genus. ***(Norris & al., 1981).***

1.3.2.4.Distribution:

B. subtilis has the ability to thrive in diverse environments, both on land and in water, suggesting that it is widespread and well-suited to grow in a variety of settings across the biosphere. Nonetheless, similar to other Bacillus species, B. subtilis can p

roduce dormant endospores that are remarkably resistant when faced with nutrient deprivation and other environmental stressors ***(Sonenshein & al,2002 / ricca & al,2004)***

1.3.2.5.Pathogenicity:

B. subtilis is typically not considered a primary pathogen in humans, although it has been isolated in a number of human infections. These infections, such as bacteremia, endocarditis, pneumonia, and septicemia, have been observed mainly in immunocompromised patients. In order for infection to occur, there must first be immunosuppression of the host followed by a high dose inoculation of B. subtilis. Additionally, there have been reports of food poisoning caused by B. subtilis contaminated food. B. subtilis does not typically produce significant quantities of extracellular enzymes or other factors that would predispose it to cause infection,

and unlike several other species within the Bacillus genus, B. subtilis is not known to be toxigenic. However, B. subtilis does produce the extracellular enzyme subtilisin, which has been associated with allergic or hypersensitivity reactions in individuals who are repeatedly exposed to it**? (*Can Bacillus subtilis cause food poisoning – Newsbasis.com)***

1.3.3.*Pseudomonas aeroginosa*:

1.3.3.1.Definition:

Pseudomonas aeruginosa is a type of bacteria that is commonly found in the environment and has the ability to infect not only humans but also animals and plants. It is considered an opportunistic pathogen, meaning that it can cause infections when the host's immune system is compromised. In humans, P. aeruginosa can cause a variety of infections, including wound and burn infections, as well as respiratory infections, particularly in individuals with cystic fibrosis. Due to its ability to form biofilms and produce multiple virulence factors, it can infect multiple host species. P. aeruginosa is also known for its antibiotic resistance, which is primarily attributed to its efflux systems and antibiotic modifying enzymes ***(Stover CK & all 2000*)** This bacterium is facultative aerobes that grow with aerobic and anaerobic respiration, ***(Diggle, S. P., & Whiteley, M. (2020).***

1.3.3.2. Taxonomy:

Table 1.11: Taxonomy of *Pseudomonas aeruginosa* (**Classification of pseudomonas according to integrated taxonomic information system (ITIS) (*Palleroni 2015*).**

Pseudomonas aeruginosa	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	Pseudomonas

1.3.3.3.Morphology:

P. aeruginosa is a type of bacteria that is characterized by its bacillus shape and gram-negative properties. Its size ranges from 0.5 to 3.0 µm, and it has an aerobic metabolism. The bacterium possesses a single flagellum that aids in its movement. P. aeruginosa is considered a non-fermentative bacterium, and in the presence of oxygen, it utilizes the glycolytic pathway to degrade glucose, with oxygen acting as the final electron acceptor ***(Ramos, J.-L. 2011)***

P. aeruginosa is a versatile species that has the ability to survive in a diverse range of environmental conditions, mainly related to chemical composition. It can utilize a variety of

carbon, nitrogen, and energy sources. However, the bacterium has a restricted range of growth temperatures ***(Palleroni, N.J.,2010)*** with the optimal temperature being 37◦C. Nonetheless, P. aeruginosa can tolerate temperatures between 4 and 40◦C, indicating its ability to adapt to a broad temperature range **(*Clark, S.T & all,2015)***

1.3.3.4. Distribution:

Pseudomonas aeruginosa is commonly found in freshwater and other environmental sources. In urban communities, it can be found in swimming pools and other bodies of water. This bacterium is responsible for a variety of infections that are acquired in the community, such as pneumonia, folliculitis, otitis externa, and osteomyelitis, often as a result of puncture wounds. P. aeruginosa is an opportunistic pathogen, meaning it usually infects individuals with compromised immune systems. It is also a significant cause of infections that occur in healthcare settings, including ventilator-associated pneumonia and urinary tract infections related to catheter use. In hospitals, P. aeruginosa can be found in a variety of reservoirs, including taps, drinking water, sinks, ice makers, toothbrushes, disinfection solutions, disinfectants, respiratory therapy equipment, soap bars, and endoscope washers **(Diggle SP & all 2020 / Kerr KG & all 2009/ Mulcahy LR & ALL 2014).**

1.3.3.5. Pathogenicity:

Pseudomonas aeruginosa is a common bacterium found in the environment, particularly in freshwater, and can also be found in urban areas such as swimming pools. It has the potential to cause a variety of infections in the general population, including pneumonia, folliculitis, otitis externa, and osteomyelitis, often as a result of puncture wounds. As an opportunistic pathogen, it can also cause infections in hospital settings, such as ventilator-related pneumonia and catheter-related urinary tract infections. In hospitals, P. aeruginosa can be found in a variety of reservoirs, including taps, sinks, ice makers, toothbrushes, disinfection solutions, disinfectants, respiratory therapy equipment, soap bars, and endoscope washers ***(Diggle SP, & all 2020 / Kerr KG & all 2009 / Mulcahy LR & all 2009)***

1.3.4. *Staphylococcus aureus:*

1.3.4.1.Definition:

Staphylococcus aureus (S. aureus) is a gram-positive bacterium that is commonly found in the microbiota of about 30% of healthy individuals across various body sites ***(Oliveira D & all 2018)*** However, it can also cause a range of infections, from mild to life-threatening, in both hospital and community settings ***(Oliveira D & all 2018/ Taylor TA & all 2020)*** .S. aureus relies on the expression of various virulence genes, including those that encode toxins and enzymes, to cause infections. Moreover, the virulence of S. aureus has been heightened by the emergence of antibiotic-resistant strains **(*Oliveira D & all 2018/Turner NA & all 2017).***

1.3.4.2.Taxonomy:

Table 1.12: Taxonomy of *Staphylococcus aureus* ***(Ito, T., &all ,2003)***

Staphylococcus aureus	
kingdom	bacteria
Phylum	firmicutes
Class	bacilli
Order	bacillales
Family	staphylococcaceae
Genus	staphylococcus

1.3.4.3.Morphology:

Microscopic morphology S. aureus cells are Gram-positive and appear in spherical shape They often appear in clusters that resemble bunches of grapes, as the name "Staphylococcus" suggests, derived from the Greek words for "bunch of grapes" (staphyle) and "berry" (kokkos) **(*Licitra G. Etymologia 2013)***. Scanning electron microscopy shows that the cells are roughly spherical with a smooth surface ***(Greenwood D 1972)*** and their diameter typically ranges from 0.5 to 1.0 μm Transmission electron microscopy of the cells reveals a thick cell wall, distinct cytoplasmic membrane, and amorphous cytoplasm **(*Foster T 1996).***

1.3.4.4.Pathogenicity:

The process of S. aureus infections involves five stages. They are: (1) colonization, (2) local infection, (3) systemic dissemination and/or sepsis, (4) metastatic infections (5) toxicosis. The organism is in carrier state in the anterior nares and can remain so without causing infections for weeks or months. The colonization proceeds to infection under certain predisposing factors such as prolonged hospitalization, immune suppression, surgeries, use of invasive medical devices and chronic metabolic diseases. Localized skin abscess develops when the organism is inoculated into the skin from a site of carriage. This can further spread and results in various clinical manifestations of localized infections such as carbuncle, cellulitis, impetigo bullosa or wound infection. The organism can enter into blood and spread systemically to different organs causing sepsis. This haematogenous spread may result in endocarditis, osteomyelitis, renal carbuncle, septic arthritis and epidural abscess. Without a blood stream infection, specific syndromes can occur due to extra cellular toxins of S. aureus. These are toxic shock syndrome, scalded skin syndrome and foot borne gastroenteritis ***(Archer GL. 1998)***

1.3.5.*Salmonella:*

1.3.5.1.Definition:

Salmonella bacteria are the major cause of food poisoning. These Gram-negative enteropathogenic bacteria can successfully colonize animals, humans and plants. Their genus is divided into two species: Salmonella bongori and Salmonella enterica, encompassing several hundred isolates, which are typically named after the place of origin . The species S. enterica is additionally divided into seven subspecies, one of them, S. enterica subsp. enterica, is the major

cause of salmonellosis in humans. The most common mode of infection is ingestion of contaminated food or water.

1.3.5.2. Taxonomy:

Table 1.13: Taxonomy of Salmonella (https://www.microscopemaster.com/salmonella.html)

Salmonella	
Domain	***Bacteria***
kingdom	***bacteria***
phylum	***proteobacteria***
class	***Gamma proteobacteria***
order	***Enterobacterial***
family	***Enterobacteriaceae***
Genus	***salmonella***

1.3.5.3. Morphology:

The genus Salmonella is an enteric Gram-negative, facultative anaerobic and non-spore forming bacillus with cell diameters ranging from 0.7 to 1.5 µm and lengths from 2 to 5 µm, belonged to the family Enterobacteriaceae ***(Tindall & al., 2005/ Todar, 2008).*** They are chemotrophs and mostly have peritrichous flagella except for S. Gallinarum and S. Pullorum, which are severely pathogenic to poultry and are non-motile ***(Bhunia, 2008)***.

1.3.5.4. Habitat:

The habitat of the genus Salmonella seems to be limited to the digestive tract of humans and animals. Thus, the presence of Salmonella in other habitats (water, food, natural environment) is explained by faecal contamination. Some serovars (serotypes) have a habitat limited to a host species, such as humans (serovars Typhi, Paratyphi A), sheep (serovar Abortusovis) or fowl (Gallinarum***) (Grimont & all,2000).***

1.3.6. *Candida albicans:*

1.3.6.1. Definition:

Candida albicans is the most well-studied and widely distributed fungal pathogen in humans. Candida, a fungus that grows like yeast, is "imperfect," meaning that they appear to lack a complete sexual cycle; nevertheless, it is clearly possible that C. albicans and several related species participate in "parasex" (see below). C. albicans, considered obligately diploid, can form true filaments in addition to budding yeast and pseudohyphae (elongated yeast cells) found in other Candida species and the model yeast Saccharomyces cerevisiae hyphae ***(Berman, J. 2012).***

Candida albicans is an important opportunistic fungal pathogen in humans, responsible for 6.8% of hospital-acquired infections in the US ***(hidron & al,2008)***

1.3.6.2. Taxonomy:

Table 1.14: Taxonomy of *Candida albicans* **(*Sahara M.G & al,2019*)**

Candida albicans

Superkingdom	Eukaryote
Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Subclass	Saccharomycetidae
Order	Saccharomycetales
Family	Debaryomycetaceae
genus	Candida
Species	albicans

1.3.6.3.Morphology:

C. albicans grows in several different morphologies: by budding to form oval yeasts and elongated pseudohyphae; or by fully polarized growth to form mononucleate mycelium similar to filamentous fungal mycelium. The phenotypic change of homozygous elliptical mating-type leukocytes to an elongated 'opaque' shape involves a switch in mating competence as well as changes in cell wall properties that are thought to make cells more permeable to mating pheromones. Another morphological switch results in the formation of enlarged, rounded "chlamydospores" at the ends of elongated stem cells in response to certain cues and nutritional conditions ***(Berman, J. (2012)).***

The ability to switch between yeast, hyphal, and pseudohyphal forms is generally considered to be required for virulence, although formal evidence is still lacking. Both hyphae and pseudohyphae are invasive ***(Sudbery, P., Gow, N., & Berman, J. (2004))***

1.3.6.4. Distribution:

Candida albicans has been isolated from a variety of warm-blooded animals and is usually present in the digestive tract. carmo susa ***(do Carmo-Sousa, L. 1969)*** considers believes that the bacteria Candida albicans and Candida stellatoidea are obligatory animal companions. Only the association with humans will be taken into account for the purposes of this review ***(odds.f.c.1979)*** has organized the pertinent epidemiological information that has been published since 1960 into a table. C. albicans is widely distributed across the globe and is typically found in the oral cavity and digestive tract of healthy individuals without causing harm. It is less commonly found in the vaginal tract of healthy women. However, its incidence is generally higher in groups receiving medical treatment, with an average occurrence of 42.9% in the oral cavity, 22% in the ano-rectal tract, and 20.7% in the vagina (excluding women with vaginitis). In the United States, Candida vaginitis is the second most common form of vaginal infection, occurring slightly less often than nonspecific vaginitis. A significant proportion of women who develop vaginal candidosis suffer from frequent recurrences or exhibit chronic and stubborn symptoms ***(Shepherd, M. G., Poulter, R. T. M., & Sullivan, P. A. (1985))***.

1.3.6.5.Pathogenicity:

Candidosis is predominantly caused by C. albicans, although other species such as C. parapsilosis and C. tropicalis can also be responsible, albeit less frequently. The disease can manifest in two forms: superficial (mucosa) and invasive (disseminated). C. albicans is capable of infecting nearly all human tissues, but superficial lesions of mucous surfaces are the most common manifestation of candidosis. In many cases, the more severe invasive form results from the spread of yeast from a mucosal focus, although yeasts can also enter the bloodstream directly through contaminated intravenous lines. The increased incidence of candidosis, both superficial and invasive, over the last two decades is well-documented and is largely attributed to the widespread use of antibiotics and immunosuppressive agents ***(Shepherd, M. G., Poulter, R. T. M., & Sullivan, P. A. (1985)).***

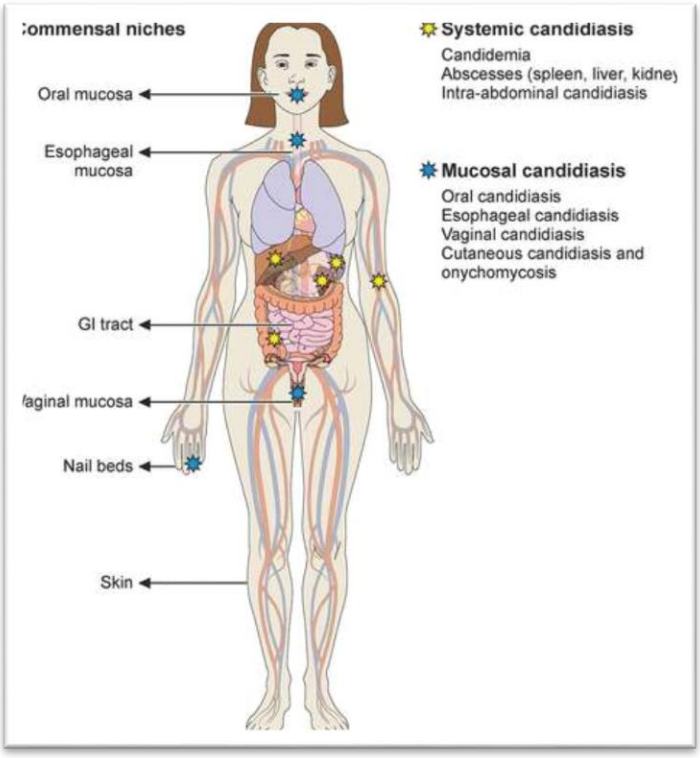


Figure 1.13: Commensal sites of *C. albicans* in the human body and clinical manifestations of *C. albicans* infection. Taking advantage of its commensal niches in the oral and genital mucosal surfaces and gastrointestinal tract, *C. albicans* can cause invasive disease (yellow star) and mucosal disease (blue star) in several tissues. Illustration created with BioRender.com.***(Lopes, J. P., & Lionakis, M. S. (2022)).***

1.3.7. *Aspergillus brasiliensis*:

1.3.7.1. Definition:

Filamentous fungi belonging to the genus Aspergillus are frequently encountered in diverse habitats such as soil, decaying vegetation, and seeds and grains, where they flourish as saprophytes. While Aspergillus species are commonly harmless, some can pose occasional risks to human health **(*Pitt JI 1994- Seyedmousavi S & all 2015)***. The majority of Aspergillus species exhibit a widespread distribution, adapting to various environments and substrates throughout

the year ***(Kwon-Chung KJ & all 2013)***. However, only a small number of well-known species are recognized as significant opportunistic pathogens in humans ***(Pitt JI 1994 / Heitman J 2011).***

1.3.7.2.Taxonomy:

Table 1.15: Taxonomy of Aspergillus **(https://acir.aphis.usda.gov/s/cird-taxon/a0ut0000002iH1LAAU/aspergillus-brasiliensis)**

Aspergillus	
Kingdom:	*fungi*
phylum	*Ascomycota*
class	*Eurotiomycetes*
order	*Eurotieles*
Family	*Aspergillaceae*
Genus	*Aspergillus*

1.3.7.3.Pathogenicity:

Aspergillus species can give rise to a wide range of human ailments, which can vary depending on the immune status of the individual **(*Denning, D. W., 1998/ Latge, J. P., 1999*).** In individuals with compromised lung function, such as asthma and cystic fibrosis patients, aspergilli can trigger allergic bronchopulmonary aspergillosis, characterized by a hypersensitive response to fungal components. In some cases, noninvasive aspergillomas may develop after repeated exposure to conidia, particularly targeting preexisting lung cavities like healed lesions in tuberculosis patients. Among the Aspergillus-related diseases, invasive aspergillosis (IA) is considered the most devastating, primarily affecting severely immunocompromised patients. Those at highest risk for this life-threatening condition include individuals with hematological malignancies such as leukemia, solid-organ and hematopoietic stem cell transplant recipients, as well as patients on prolonged corticosteroid therapy commonly used for preventing and treating graft-versus-host disease in transplant patients. ***(Kliasova, G. A & all 2005 / Marr, K. A & all 2002/ Mikulska, M., & all 2009/ Pagano, L & all 2001 / Wiederhold, N. P.,& all 2003).***

Chapter 2
Material and Methods

2.1. Periode and study place :

Our work was conducted within the laboratory of the Faculty of Sciences at the University of Médéa and Microbiology and Physicochemical block of the SAIDAL affiliate companies ANTIBIOTICAL unit located in Médéa during the period from febraury to may 2023.

SAIDAL is a generalist Algerian Pharmaceutical roup whose main mission is to produce and develop and market pharmaceutical products for human and veterinary use.

2.2. Objectif of our study :

- ✓ -The evaluation of the antimicrobial activity of all extracts from each plant used.
- ✓ -the evaluation of the antimicrobial activity of two different essential oils from *Brocchia cenirea and Artemisia herba alba* .
- ✓ -The identification of different components of the plant through phytochemical tests.
- ✓ -Formulation of a cream based on hydroethanolic extract of aloe vera.
- ✓ -Formulation of a cream based on pure gel of aloe vera .
- ✓ -The Evaluation of the wound healing and burn .

2.3. Plant material :

In this study, the plant material used consists of leaves from medicinal species *Artemisia herb alba ,brocchia cenirea ,moringa oleifera* and the gel of *aloe vera* , These species were chosen due to their availability and common use in traditional medicine and the agro-food industry.

This plant was harvested from different regions in Algeria on different periods :

Table 2.1 : Plants material

Plantes	The region	period of harvested
Moringa oleifera	Adrar	27 January
Brocchia cenirea	Adrar	1 Febraury
Artemisia herba alba	Tamanrasset	1 Febraury
Aloe vera	Nursery plant	2 March



Aloe vera ***Moringa oliefera*** ***Artemisia herba alba*** ***Brocchiacenirea***

Figure 2.1 : The four plants species **(personal photo , 2023)**

2.4. Laboratory material :

Table 2.2 : Material table

Material		
beaker	test tube	balance
pipette	round-bottom flask	stove
hot plate	separatory funnel	rotary evaporator
erlenmeyer	spatula	watch glass
flask	pissette	stand
graduated cylinder	sample carrier	Evaporating dish
Petri dish	mortar and pestle	Filter paper
funnel	magnetic stirrer	Bunsen burner
Clivenger apparatus	Autoclave	Micropipette
Colony counter	Vortex mixer	Filtration apparatus
Syringe	Blank disk	Blank disk of laboratory
Culture medium	Graduated cylinder	Ph metre

2.5. Consumable products :

Table 2.3 : Consumable products

products	
Methanol	ammonia
Ethanol	Fe cl3
Distilled water	Hydrochloric acid
Diode I2	potassium iodide
Naoh	chloroforme
Sulphuric acid	/

2.6.The culture media used:

- The culture media Soy agar .
- The culture media sabouraud .

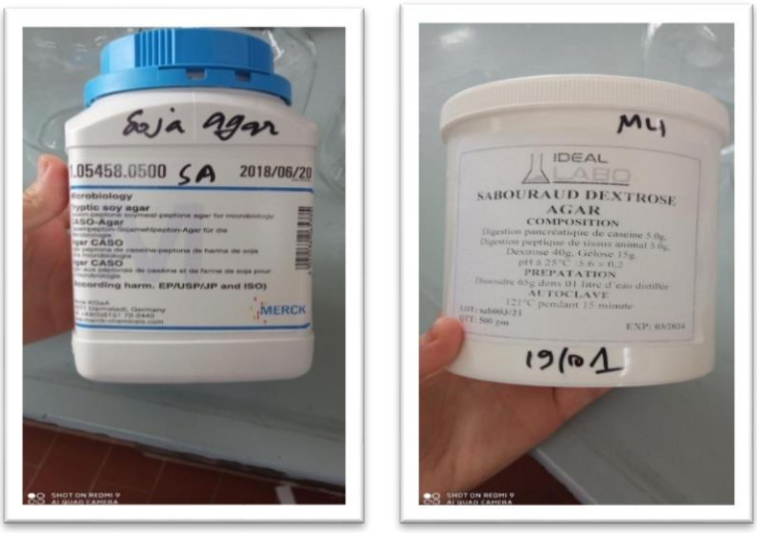


Figure 2.2 : Media culture **(personal photo ,2023)**

2.7.Microbial strains:

The strains used include five bacterial strains and two fungal strains that were selected for their high pathogenicity and multi-resistance to antibiotics. These strains were provided to us by the SAIDAL laboratory in Médéa .

Table 2.4 *:* The reference bacterial strains used**.**

Gram	Strains	Reference
Gram negative	*Pseudomonas aeruginosa*	ATCC 9027
Gram positive	*Staphylococcus aureus*	ATCC 6538
Gram positive	*Bacillus subtilis*	ATCC 6633
Gram negative	*Salmonella typhimurium*	ATCC 14028
Gram negative	*Echerichia coli*	ATCC 8739

Table 2.5 : The reference fungal strains used

Starins	Reference
Candida albican	ATCC 10231
Aspergillus brasiliensis	ATCC 16404

2.8. Methodes :

2.8.1. The plant harvesting process:

The plant material used in this study *moringa oliefera . it* was collected in the month of janaury from the regions of the adrar province in south of Algeria.

The plant material used in this study *Artemisia herba alba*, it was collected in the month of febraury from the regions of the tamanrasset province in south of Algeria.

The plant material used in this study *brocchia cenirea* , it was collected in the month of febraury from the regions of the adrar province in south of Algeria.
The plant material used in this study *aloe vera* , was collected in the month of march from the regions of the blida in a nursery plant province in north of Algeria.

2.8.2. Sample preparation:

2.8.2.1. Drying and grinding:

The collected plant material was sorted, rinsed, and dried in the shade at room temperature for 1 week. Once the plant material was dry, the leaves of each plant were ground using a mortar and then subjected to extraction.

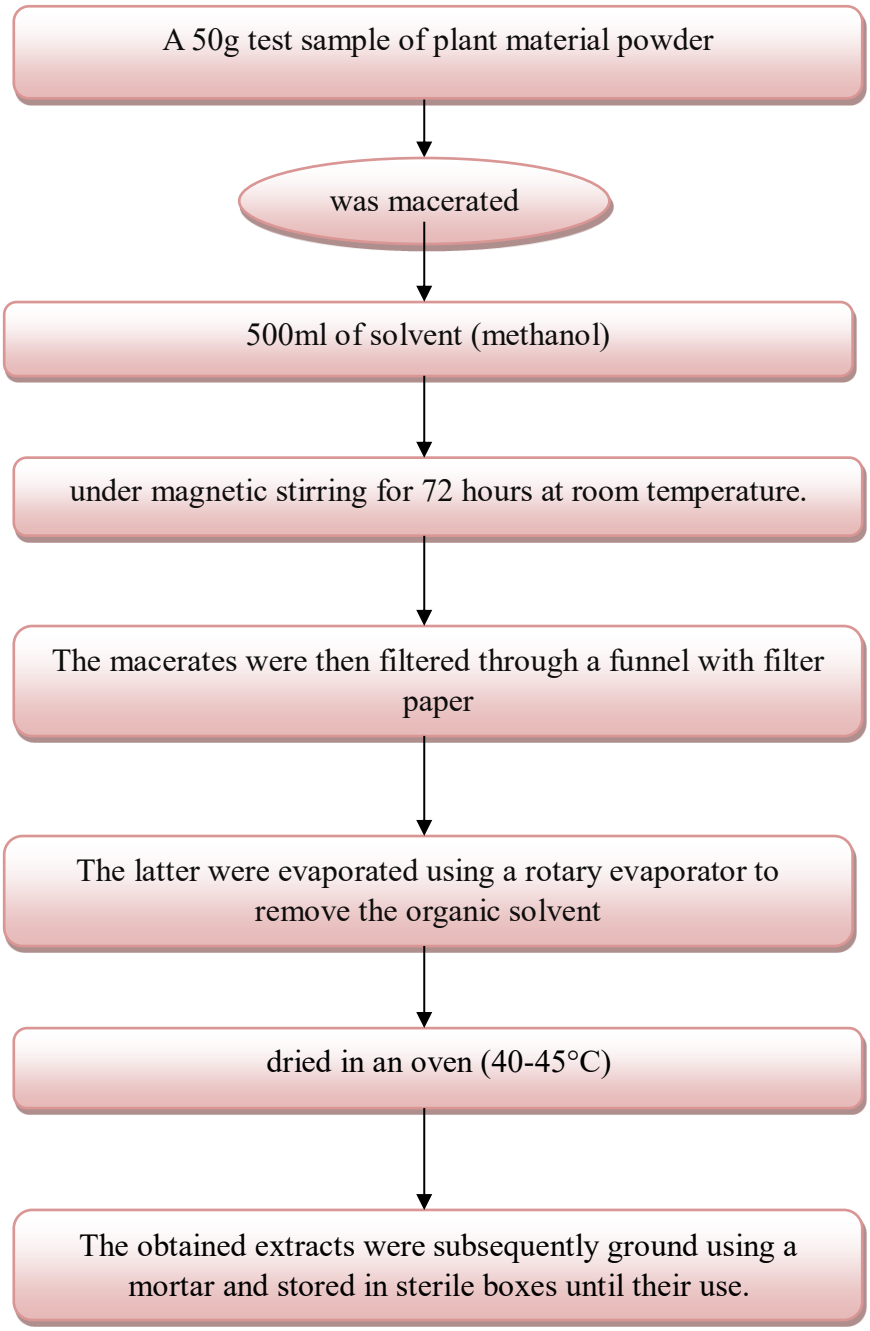
2.8.2.2.Extraction of aloe vera gel :

- Select mature and healthy aloe vera leaves .
- Wash the leaves with distilled water to remove the dirt .
- Cut off the spiky edges of the leaves .
- Make a longitudinal incision along the length of the leaf exposing the inner gel .
- Use a spatula to scoop out the gel from the leaf.
- Collect the gel in a clean container.
- Blend the gel in a blender to obtain a smooth consistency.
- Store the extracted aloe vera gel in a clean container in the refrigerator for future use.

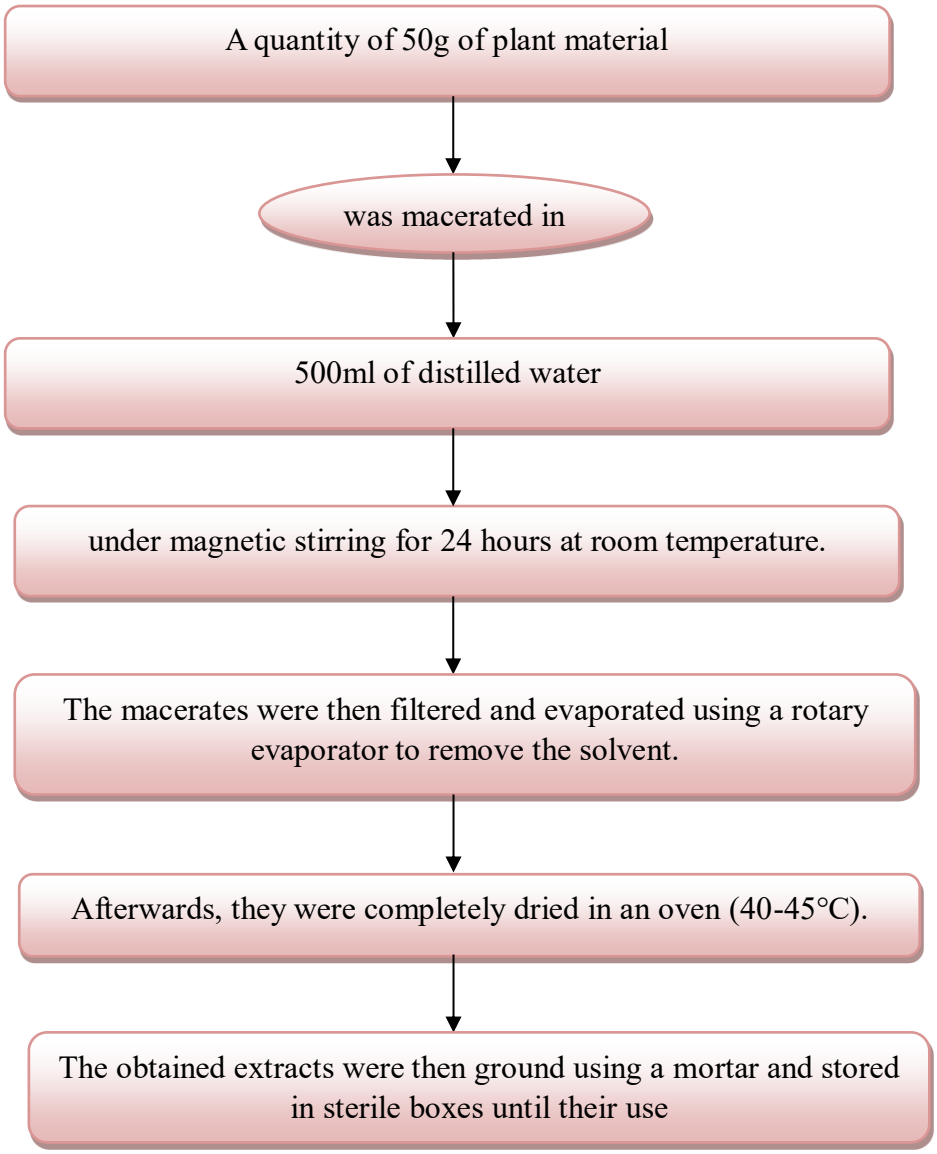
2.8.3. The extraction method:

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures **(Handa ss & all,2008).** The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular (residue),The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing **(azwanida, N & all,2015).**

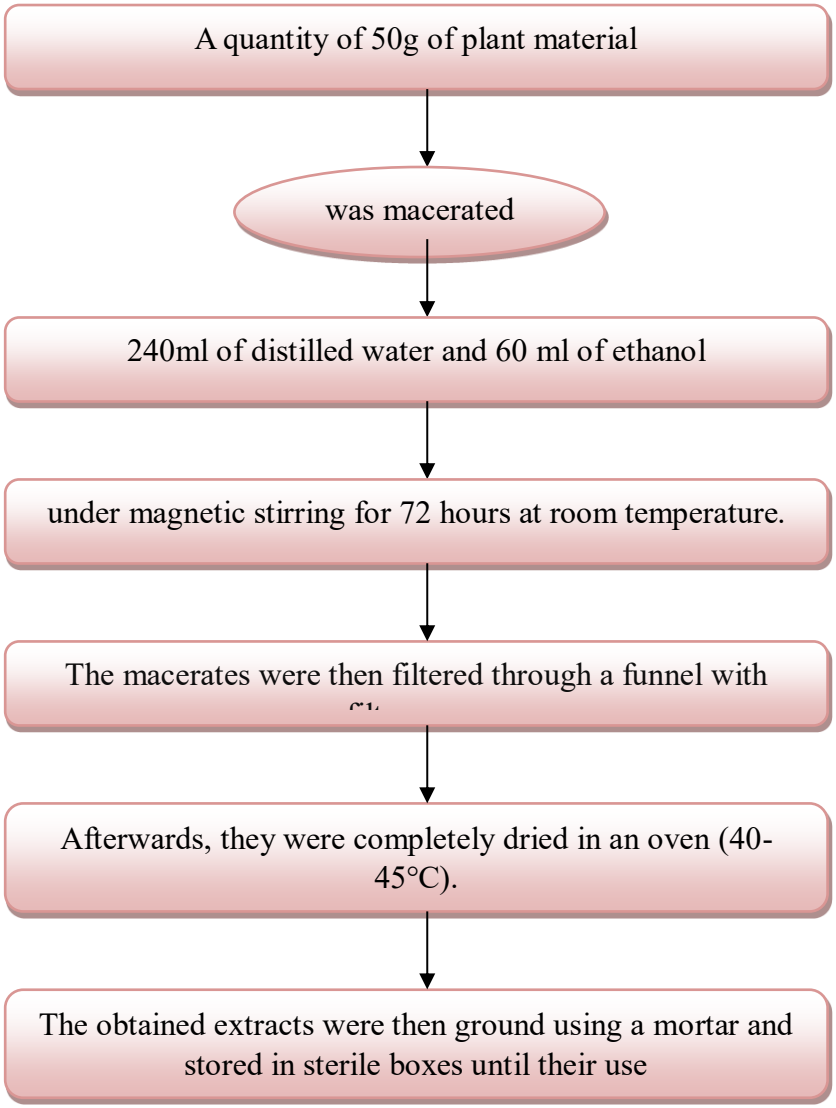
2.8.3.1. Preparation of methanolic extract:

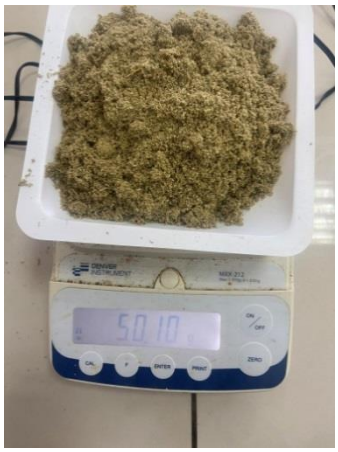


2.8.3.2. Preparation of aqueous extracts:

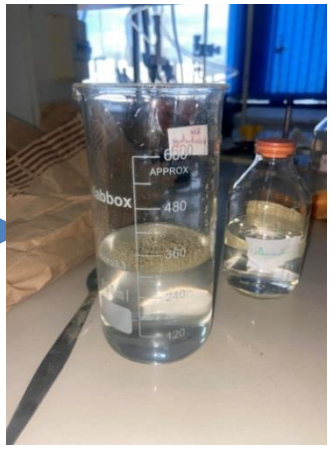


2.8.3.3. Preaparation of hyrdroethanolic extract :

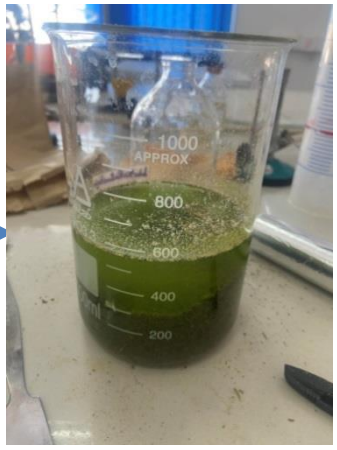




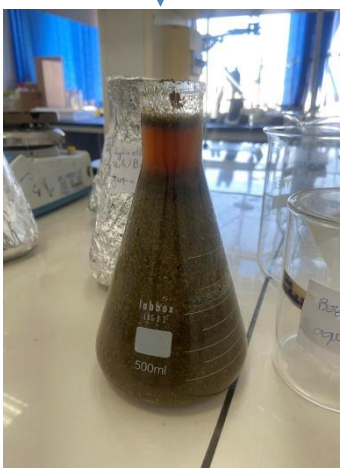
A



B



C



D



E



F



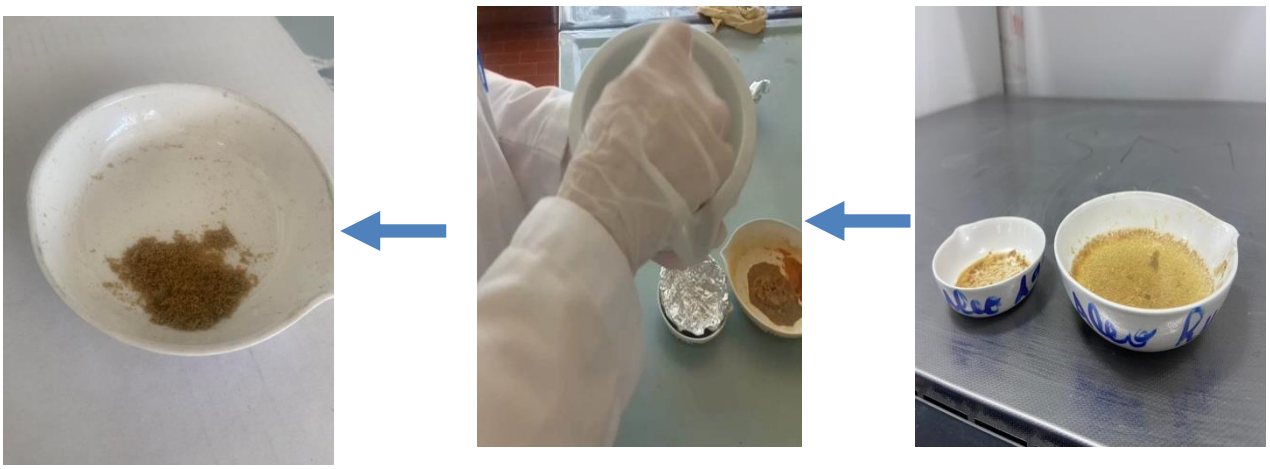
G



H



I



L **K** **J**

Figure 2.3: The process of extraction (aqueous/ hydroethanolic/methanolic**)(personal photo,2023)**

A/ 50f of vegetable powder was weighed , C/D:plant powder was mixed with methanol (methanolic extract) and distilled water for the aqueous extract, and with ethanol and water for the hydroethanolic extract , E/ magnetic agitation, F/ filtration , G/ evaporation ,H/ pored in evaporating dish , I / dryed in the over 40° 45° , J / the extract was dryed , K/ grinding , L / powder extract .

2.8.4. Detramination of the yield of the extracts :

The determination of the yield of plant extracts provides valuable insights into the effectiveness of extraction processes, supports quality control measures, and aids in understanding the potential therapeutic value of and natural compounds, The yield of this extracts were calculated by the following equation:

$$\text{the plant extract (\%)} = \frac{the\ extract\ (g)}{50g\ of\ plant\ material} * 100$$ *(onzo C.F 2015)*

2.8.5. Extraction of essential oils:

50 grams of plant material (*Artemisia herba alba, brocchia cenirea*) were placed in a round-bottom flask of a clevenger, along with 500 ml of distilled water. The mixture was brought to a boil for approximately 3 hours. The vapors were condensed in a condenser. The collected liquid resulted in a distillate with a thin layer of oil on the surface, which would be separated after allowing the liquid to settle. As a result, the two extracts (essential oil and hydrosol) were obtained separately. The yield of this essential oil were calculated by the following equation :**(singh et all.2013)**

$$\text{Essential oil yield (\%)} = \frac{extracted\ essential\ oil\ (g)}{50g\ of\ plant\ material} * 100$$

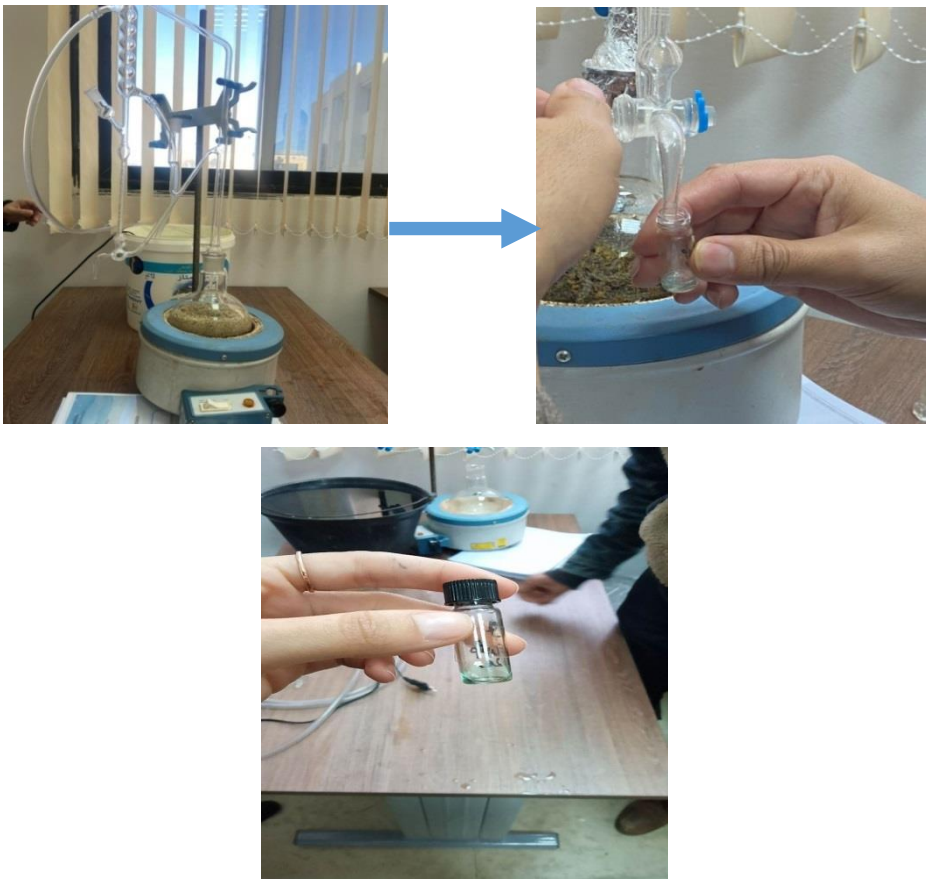


Figure 2.4: Extraction of essential oils **(personal photo,2023)**

2.9. Phytochemical study :

2.9.1.Phytochemical screening :

Phytochemical screening is a method used to highlight the presence of chemical groups and families in plants studied carried out on the powder of three plants and the gel of *aloe vera* , this work was conducted at the physico chemical laboratory of saidal medea unit under biosafety cabinets.



Figure2.5: biosafety cabinets of physic chemical department of SAIDAL unit medea **(photo personal, 2023)**

2.9.2. Operating procedure:

This test is qualitative and is performed on an infusion. It constitutes the first step in the search for molecules with therapeutic activities, involving coloration and precipitation reactions. This study allows for the identification of the presence of various chemical groups in plantes.

2.9.2.1. Protocol:

- **Preparation of infusion:**

10 g of plant poudre mixed with 50 ml of distilled water and we put it on fire until boiling about 15 minutes. after that the infused was filtered .

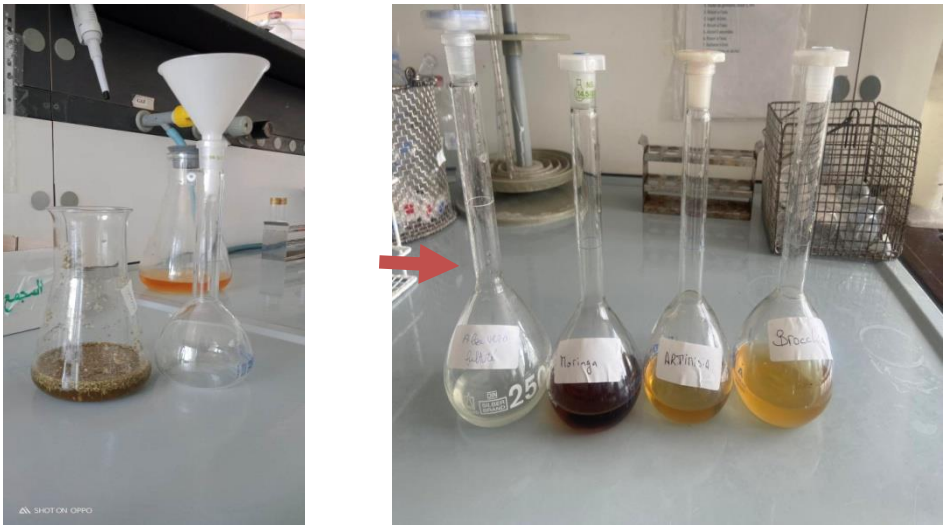


Figure 2.6 : Preaparation of the infused **(photo personal, 2023)**

- **Detection of anthocyanes:**

5 ml of infusion are added with a few drops of ammonia, the appearance of a red color indicates the presence of anthocyanins.

- **Detection of tannins:**

5 ml of infusion are added with a few drops of 5% FeCL3 solution, the reaction produces a dark blue coloration in the presence of tannins.

- **Preparation of FeCL3 :**

5g of feCL3 powder in 100 ml of distilited water.



Figure 2.7 : Preparation of feCL3

- **Detection of mucilages :**

1ml of the infusion is introduced into a tube and 5 ml of absolute ethanoole is added. the formation of precipitation indicates the presence of mucilages.

- **Detection of flavinoides :**

In a test tube containing a quantity of the infusion, a few drops of sodium hydroxide (NaOH) solution are added. The formation of an intense yellow color that becomes colorless after the addition of diluted hydrochloric acid (HCl 2N) reveals the presence of flavonoids.

- **Detection of glucosides :**

To 2g of plant powder, a few drops of sulfuric acid (H2SO4) are added. The formation of a reddish-brown color, followed by a violet coloration, indicates the presence of glucosides.

- **Detection of quinones :**

2g of plant powder moistened with 1N HCl are in contact for 3 hours in 20 ml of chloroform, then filtered. The filtrate is then shaken with 5ml of ammonia ½. The formation of a red coloration indicates the presence of free quinines.



Figure 2.8 : Preparation of tubes for phytochemical screening **(photo personal ,2023)**

- **Detection of alcaloides :**

- **Preparation of solution :**

1g of powder is added to 100 ml of ethanol, then agitated and filtered to obtain 100 ml of solution for analysis .



Figure 2.9 : Preparation of solution **(personal photo ,2023)**

- **Protocol :**

The detection of alkaloids involves treating the analyzed solution with another solution formed by 0.16 g of potassium iodide (KI) and 0.015 g of iodine (I2) in 10 ml of distilled water. A reddish-brown coloration indicates the presence of alkaloids in the sample to be tested.

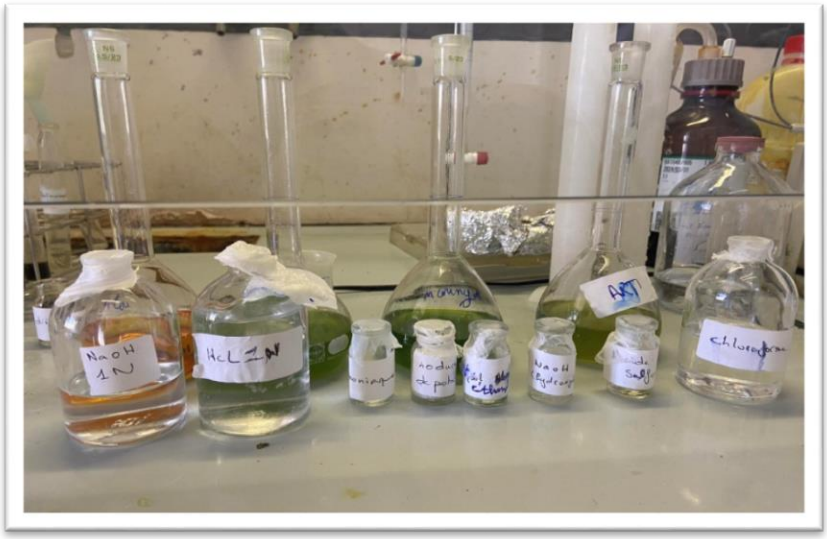


Figure 2.10 : The products used in phytochemical analysis

2.10. The evaluation of antibacterial activity and antifungal activity:

2.10.1. Antimicrobial activity:

The evaluation tests of antimicrobial activity are conducted at the quality control laboratory, microbiology department of SAIDAL -Médea. This test must be carried out under strict aseptic conditions to avoid contamination issues. Additionally, materials, solutions, and culture media must be sterilized using an autoclave.

The evaluation of antimicrobial activity was conducted using the disk diffusion method (aromatogram) to test the sensitivity of the strains, specifically seven reference microbial strains from the American Type Culture Collection (ATCC).There are five bacterial strains three Gram-negative and two Gram-positive) involved: *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 14028, *Bacillus subtilis* ATCC 6631, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739. Additionally, there are two fungal strains known to be responsible for nosocomial infections: *Candida albicans* ATCC 10231and *Aspergillus brasiliensis* ATCC 16404.,

2.10.2.The Culture media:

-The culture media used were as follows:

Soy Agar (TSA): is a general purpose plating medium used for the isolation, cultivation, and maintenance of a variety of fastidious and nonfastidious microorganisms, is also a highly nutritious base that meets the growth requirements of many types of microorganisms including bacteria, yeasts, and molds.
(https://www.dalynn.com/dyn/ck_assets/files/tech/PB81.pdf)

Sabouraud agar: is a selective medium for fungal culture and primarily used for isolating dermatophytes, yeasts, and various other pathogenic and non-pathogenic fungi. (https://microbiologie-clinique.com/gelose-sabouraud.html)



Figure 2.11 : Preparation of media culture

2.10.3. Preparation of inoculum:

The different bacterial species are revived by successive passages on appropriate agar media to obtain a culture of 18 to 24 hours. Then, 4 to 5 well-isolated bacterial colonies are suspended in 0.9% NaCl physiological water. This suspension is then adjusted to a 0.5 McFarland turbidity standard.

2.10.4. Preparation of dilutions:

2.10.4.1. Extract dilution :

An initial solution (200 mg/ml) of the studied extract was prepared using an organic solvent, DMSO. From this stock solution, half-dilutions were made in a concentration range from 200 mg/ml , 100 mg:ml , 50mg/ml ,25 mg/ml.



Figure 2.12 : The dilutions **(personal photo 2023)**

2.10.4.2. Essential oils dilution :

An initial solution (8mg/ml) of the studied essienstial oil was prepared using an organic solvent, DMSO. From this stock solution, half-dilutions were made in a concentration range from 8 mg/ml , 4 mg:ml , 2mg/ml ,1 mg/ml

2.10.5. The inoculation of bacteria:

After the culture media have cooled and solidified, a sterile swab soaked with a freshly prepared bacterial suspension of approximately 10^8 CFU/ml is streaked into the agar surface three times. After each application, the plate is rotated about 60° without forgetting to rotate the swab itself. The final streaking is done by passing the swab along the periphery of the agar to ensure an even distribution of the inoculum. All plates are left to dry for 5 minutes.

2.10.5.1. Application of the disks

The sterile white disks with a diameter of 9mm are impregnated with the extracts (04 disk for each extract) and essential oil by bringing only the tip of the disk into contact with the extracts and essential oils, which will gradually absorb them until the disk is fully impregnated. These impregnated disks are then placed on the agar surface using sterile bacteriological forceps. The antibiotic disk (aztreonam/streptomycin) is also placed on the agar surface using sterile bacteriological forceps. The antibiotic serves as a positive control in the experiment.

2.10.5.2. Incubation:

The petri dishes were incubated at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungi.

2.11. Antifungal activity:

The evaluation of the antifungal activity of the extracts against the tested fungal strains is performed using the method of diffusion on solid medium. On a sabouraud agar .
From a freshly prepared pure culture, obtained after 48 hours at 25°C, well-isolated colonies were scraped and suspended in physiological saline solution (0.9% saline). The fungal suspension should be homogeneous, and the optical density is measured using a spectrophotometer at a wavelength of 625nm to standardize the spore suspension to a concentration of 107 spores/ml. It is estimated that an optical density of 0.08-0.10 corresponds to a concentration of 107 spores/ml.

2.11.1. The inoculation :

In Petri dishes, Sabouraud culture medium is aseptically poured at a volume of 15 ml per dish. After cooling and solidification on the bench, a sterile swab soaked with the freshly prepared bacterial suspension is streaked on the surface of the agar three times, rotating the dish approximately 60° after each application. The swab is rotated on itself and the inoculation is completed by streaking the swab along the periphery of the agar to ensure even distribution of the inoculum. All the dishes are allowed to dry for 5 minutes.

After inoculation, sterile discs with a diameter of 9mm, soaked in the extracts and the essential oils and placed on the dried agar surface. These petrie dish are left on the bench for 30 minutes to allow proper diffusion of the extract into the agar, and then transferred to an incubator at 25°C for 48 hours.



Pour the agar in a Petri dish



Allow the agar to solidify



the Inoculation



Preparation of the extract dilution



mix the extract with DMSO



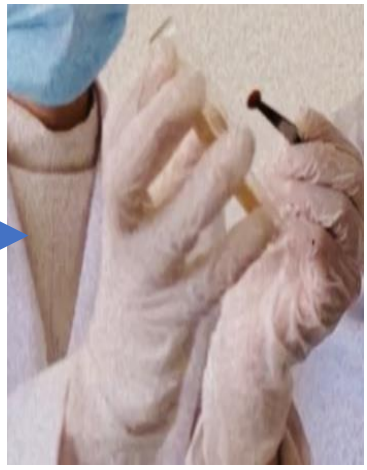
preparation of the



dilutions



impregnate the disks in the extracts



application of the discs



Distribution of impregnated discs

Figure 2.13 : the stages of antimicrobial activity (personal photos ,2023)

2.12. Determining the minimum inhibitory concentration (MIC):

After the incubation period, the value of the inhibition zone diameters, where the tested microorganisms have not grown (visible zone of inhibition), is measured using a colony counter. The sensitivity of the tested strains to the extracts and essential oil is classified based on the diameters of the inhibition zones **(ponce et all 2003)**:

-Diameter less than 8mm: not sensitive (-) or resistant.

-Diameter between 9mm to 14mm: sensitive (+).

-Diameter between 15mm to 19mm: highly sensitive (++).

-Diameter greater than 20mm: extremely sensitive (+++).

2.13. Formulation of a healing cream for burns and wounds based on pure aloe vera gel and aloe vera extract:

2.12.1. Introduction:

The aloe vera cream was manufactured at the physico chemical laboratory of SAIDAL Medea unit. The choice to embark on the formulation of a healing cream for burns and wounds is driven by the desire to offer individuals an innovative, natural, and effective treatment option. By tailoring the formulation to address the unique characteristics of these injuries and incorporating well-known healing ingredients, it is hoped that the resulting ointment will provide individuals with a reliable and accessible solution for managing burns and wounds, promoting optimal healing, and restoring their well-being.

we've choosed *aloe vera* gel for their therapeutic properties and ability to aid in wound healing. The decision to incorporate these ingredients into the formulation stems from their

documented effectiveness in promoting tissue regeneration, reducing inflammation, and providing antimicrobial benefits.

2.12.2. Operating procedure:

In this work, we formulated two creams based on almond oil. The first cream was prepared using the hydroethanolic extract (active ingredient) of aloe vera, while the second cream was formulated with pure aloe vera gel. The purpose of formulating these two creams was to see their effectiveness in treating burns and scars within a short duration.

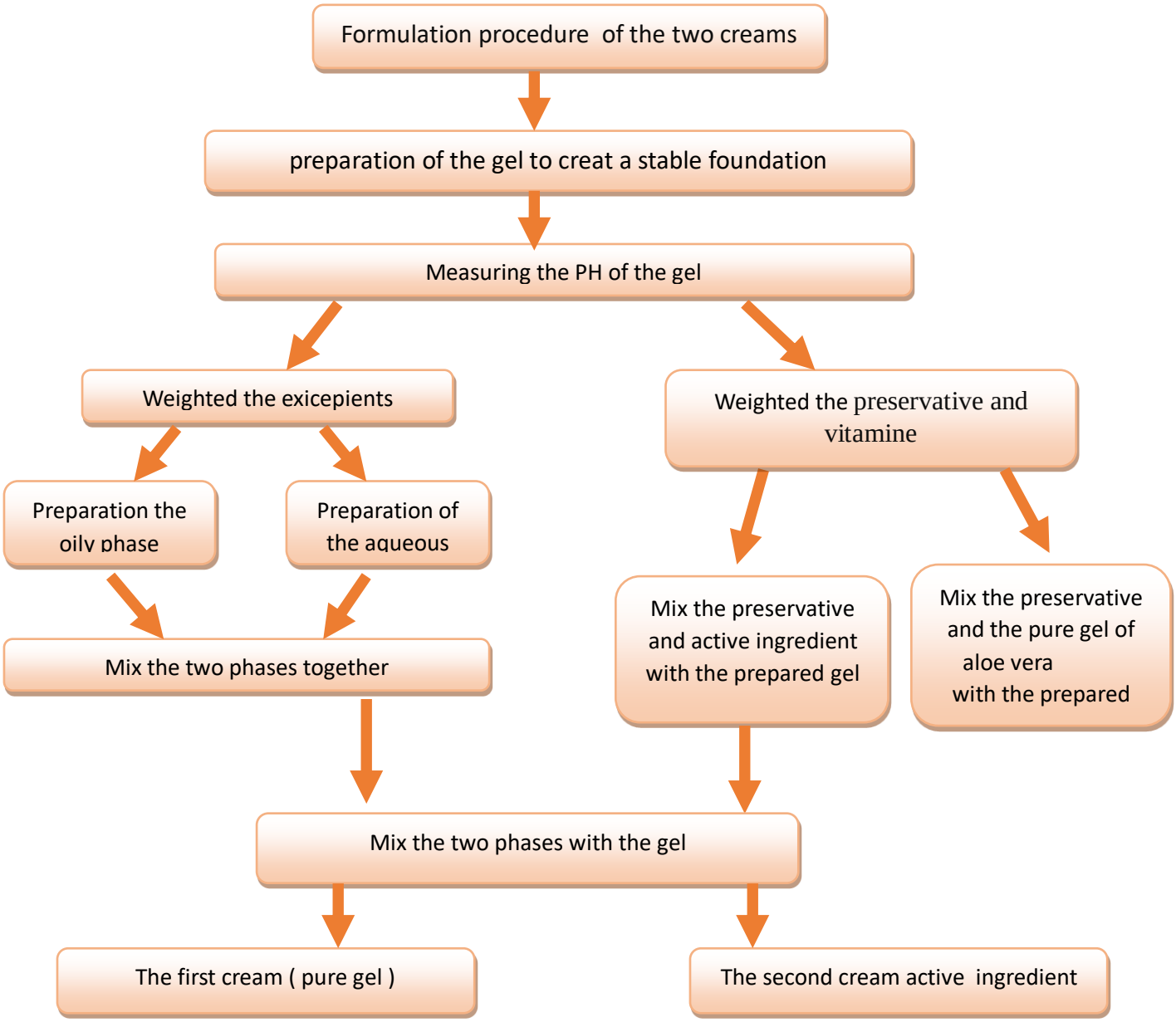


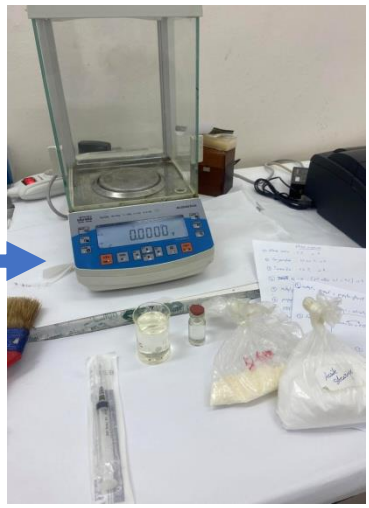
Figure 2.14 : Plan of the cream formulation



Pur gel of aloe vera



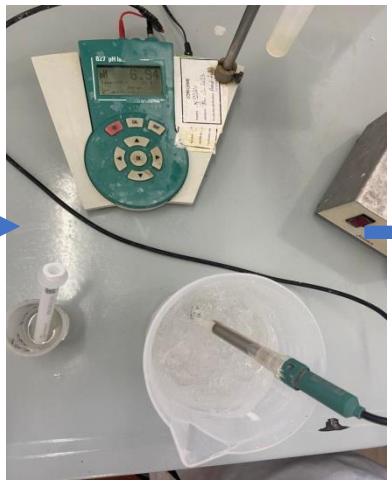
aloe vera extract



weighing of the excipients.



Preparation of gel excipients



measuring the PH of the gel



agitat the



Agitation of preservative



mix the cream



the mixture



Added Vitamine **the first cream of pure gel** **the second cream (active ingredient)**

Figure 2.15 : The Formulation procedure for the two creams

2.13. Study of wound and burn activity :

This work is carried out at the laboratory of the univertisy Yahia Fares Medea with the help of professional professors , the two creams are tasted on four albinos rabbits , two rabbits to test the effect of the first cream and the other two rabbits to test the second cream . we tested the wound effect on two rabbits , one for the first cream and the other for the second cream , the burn test is conducted using the same method on the other rabbits .

2.13.1. Study of the wound activity :

A wound is defined as a disruption in the continuity of a body tissue **(Li W, Dasgeb B and all 2005).** The evaluation of the wound activity involves creating wounds on previously anesthetized animals (rabbits),They are represented by four male albinos rabbits with an average weight of 2254.16 g (after one week of adaptation), sourced from the same animal facility in the region of Medea .



Figure 2.16 : Albinos rabbits **(personal photo ,2023)**

2.13.1. 1.Protocol :

According to the SAIDAL protocol inspired by the european pharmacopoeia, the skin test is conducted on rabbits here are the steps of the test:

- **Shaving:**

The four animals were shaved on their right and left tights using an electric clipper and a razor blade on each side of the legs, before the application of the scratch.



Figure 2.17 : Shaving the hair **(personal photo)**

- **Disinfection:**

The shaved area of the four rabbits was then disinfected using surgical alcohol with a sterile cotton pad.



Figure 2.18 : The rabbit's disinfection using surgical alcohol **(personal photo)**

- **Anesthesia:**

All the four rabbits were anesthetized with a subcutaneous injection of (0.2ml) on each side of the rabbit tight , and the animals received local anesthesia through infiltration of 0.2ml xylocaine for each area .

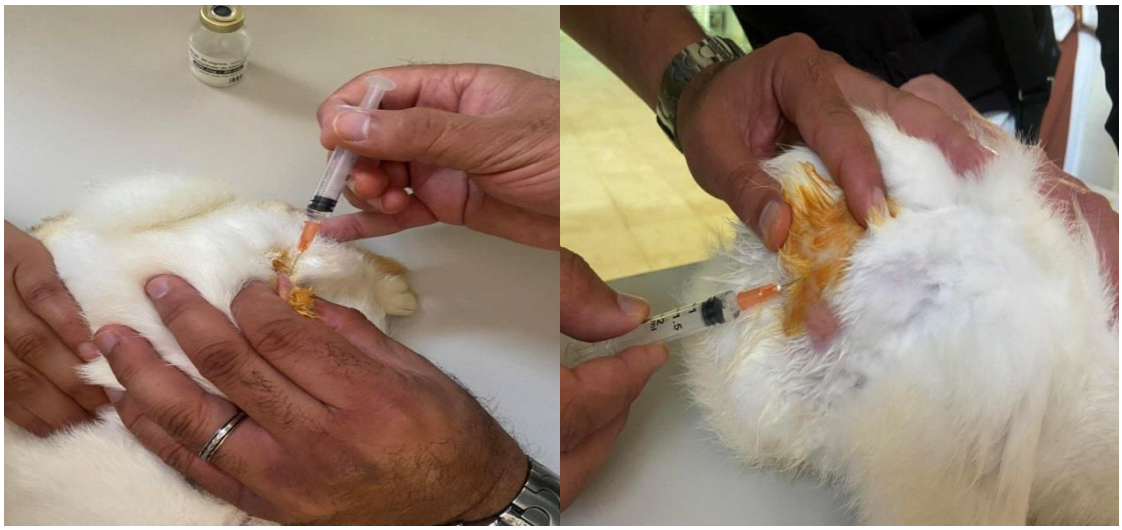


Figure 2.19 : Anesthesia for the rabbit's **(personal photo)**

2.13.1. 2.Wounds technique:

This study was conducted on two rabbits, A controlled incision is made on the skin of the rabbits using a sterile blade , make two slight scratch marks (scarfication) on each area of the rabbit's leg (3 cm)



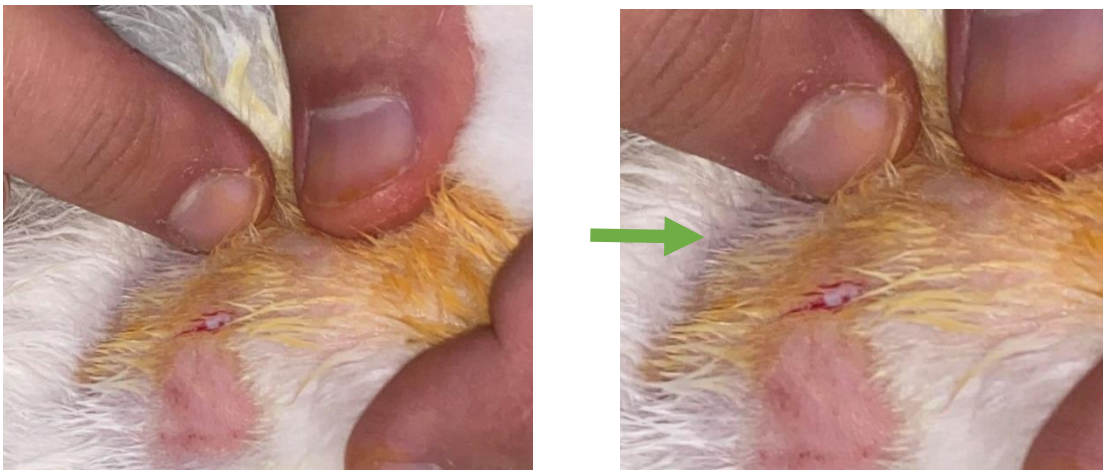


Figure 2.20 : Wounds of the second rabbit left side **(for the pure gel cream)**



Figure 2.21 : Wounds of the second rabbit right side **(for the pure gel cream)**



Figure 2.22 : Wound of the forth rabbit left side **(for principal ingredient cream)**

-Apply the first cream of aloe vera (pure gel) directly onto the scratch marks on a specific area of one side of the tight for the first rabbit , every day for a period of 15days , the second scratch of the other tight is considered a negative control , no products is applied to it .



Figure 2.23: Application of the cream (pure gel) on the left tight of the second rabbit**(personal photo)**

-Apply the second cream of aloe vera (actif ingredient) directly onto the scratch marks on a specific area of one side of the leg for the second rabbit , every day for a period of 15 days .



Figure 2.24: Application of the second cream (active ingredient) on the left tight of the forth rabbit

-During the 15 days application period make regular observation of the rabbits skin condition and make the difference between the two creams.

-Continue to observe the rabbits for 15 days after the end of product application to detect any delayed or persistent effects .

-The observation was taken every four days : day 1 , day 4 , day 8 , day 12 , day 15.

2.13.2.<u>Study of the burn activity :</u>

Burns are one of the most common household,it characterized by severe skin damage that causes the affected skin cells to die . The evaluation of the burn activity involves creating burns on previously anesthetized animals (rabbits),They are represented by four male albinos rabbits with an average weight of 2254.16 g (after one week of adaptation), sourced from the same animal facility in the region of Medea .

2.13.2. Burn technique :

This study was conducted on the other two rabbits, Burns were made on the left and right thigh of each rabbit using a metal spatula that was heated for 4 minutes under the fire and immediately placed on the rabbit's skin for 10 seconds. After the metal spatula cooled down, the other burns were created in the same manner. The severity of the burns was consistent among the groups, and their appearance indicated that the third rabbit has the third-degree burns and forth rabbit has the second degree burns,the second burns on the rights tight of the rabbits is considered a negative control.



Figure 2.25 : Burn the left tight of the first rabbit **(personal photo)**



Figure 2.26: Burn the right tight of the first rabbit **(personal photo)**

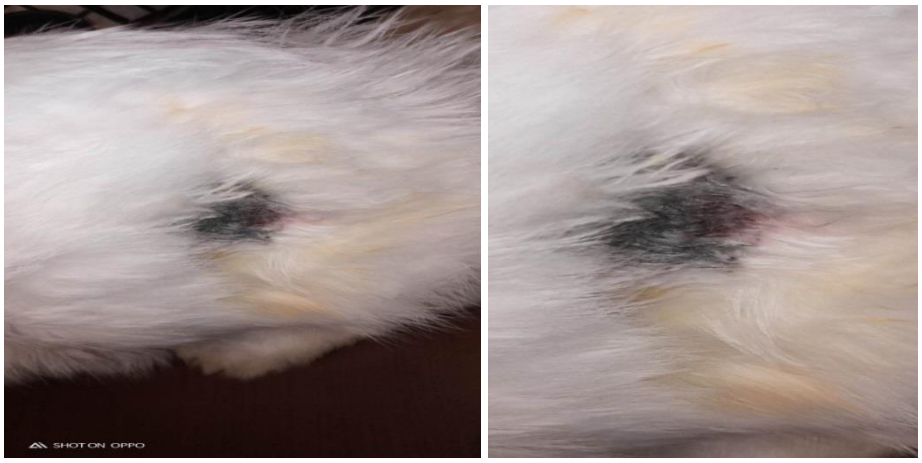


Figure 2.27 : Burn of the third rabbit left side **(personal photo)**

-Apply the first cream of aloe vera (pure gel) directly onto the scratch marks on a the left tight of the first rabbit , every day for a period of 15days , and the right tight considerd a negative Control no product is applied to it .

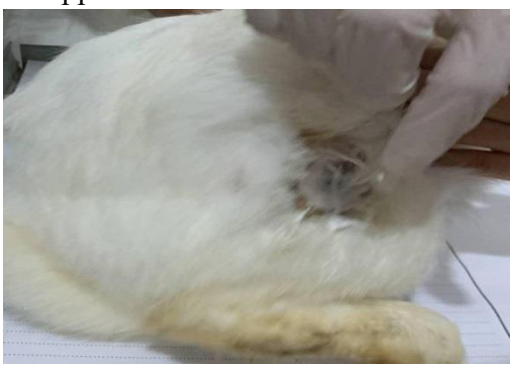


Figure 2.28 : Application of the first cream(pure gel) on the left burn tight of the first rabbit **(personal photo)**

-Apply the second cream of aloe vera (active ingerdient) directly onto the scratch marks on a specific the left tight for the second rabbit , every day for a period of 15 days . and the right tight considerd a negative vontrol no product is applied to it .

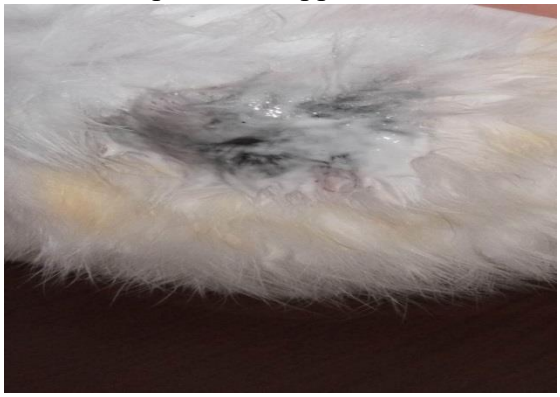


Figure 2.29 : Application of the second cream (active ingredient) in the left tight of the third rabbit **(personal photo)**

-During the 15 days application period make regular observation of the rabbits skin condition and make the difference between the two creams .

-Continue to observe the rabbits for 15 days after the end of product application to detect any delayed or persistent effects .

-The observation was taken every four days : day 1 , day 4 , day 8 , day 12 , day 15 .

Chapter 3
Results and discussion

3.1.Determination of the yield :

Table 3.1: The yield of the four plants extracts

	Methanolic extract			Aqueos extract			hydroEthanolic Extract		
	Powder weight(g)	Extract weight(g)	yield (%)	Powder weight(g)	Extract weight(g)	yield (%)	Powder weight(g)	Extract weight(g)	yield (%)
Moringa oleifera	50 gr	5,83gr	11,66 %	50 gr	7,48gr	14,96	50 gr	3,81	11,62
Brocchia cenirea	50 gr	7,27 gr	18,54 %	50 gr	10,29gr	14,58 %	50 gr	2,42gr	4,84 %
Aloe vera	50 gr			50 gr			50 gr	1,55gr	3,10 %
Artemisia herba alba	50 gr	2,51gr	5,02%	50 gr	9,04gr	18,08 %	50 gr	3,84gr	7,68 %

The obtained results showed that the aqueous extract of Moringa oleifera exhibited the highest yield (14.96%) compared to the methanolic extract (11.66%) and the hydroethanolic extract (11.62%). The methanolic extract of brocchia cenirea leaves also displayed a higher yield (18.54%) compared to the aqueous extract (14.58%) and the hydroethanolic extract (4.84%). The hydroethanolic extract of aloe vera gel had a yield of 3.10%, while the aqueous extract of artemisia herba alba exhibited the highest yield (9.04%) among all the extracts. In comparison, the methanolic extract of artemisia herba alba showed a yield of 5.02%, and the hydroethanolic extract showed a yield of 7.68%. These results highlight the variations in yield among the different studied extracts.

3.2. Phytochemical study :

The qualitative phytochemical screening of the plants powder was performed to identify the main groups of chemical constituents (glycosides, alkaloids, tannins, , glycosides, flavonoids, anthocyanes , quinones , mucilages ,) present in the extracts using the color reactions **.(Savithramma N and all 2011)**

The results of the presence or absence of different chemical groups are presented in the table :

Table 3.2: Results of phytochemical screening

	Moringa	Brocchia	Aloe vera	Artemisia
Anthocyanins	–	–	–	–
Tanin	+	+	–	+
Mucilags	+	+	+	+
Flavonoids	+	+	+	+
Glucosids	+	+	–	+
quinons	–	–	–	–
Alkaloids	–	+	+	+

➢ **Anthocyans :**

The presence of anthocyanins in the plant powder was verified using the ammonia test :

- ***Moringa oleifera :***

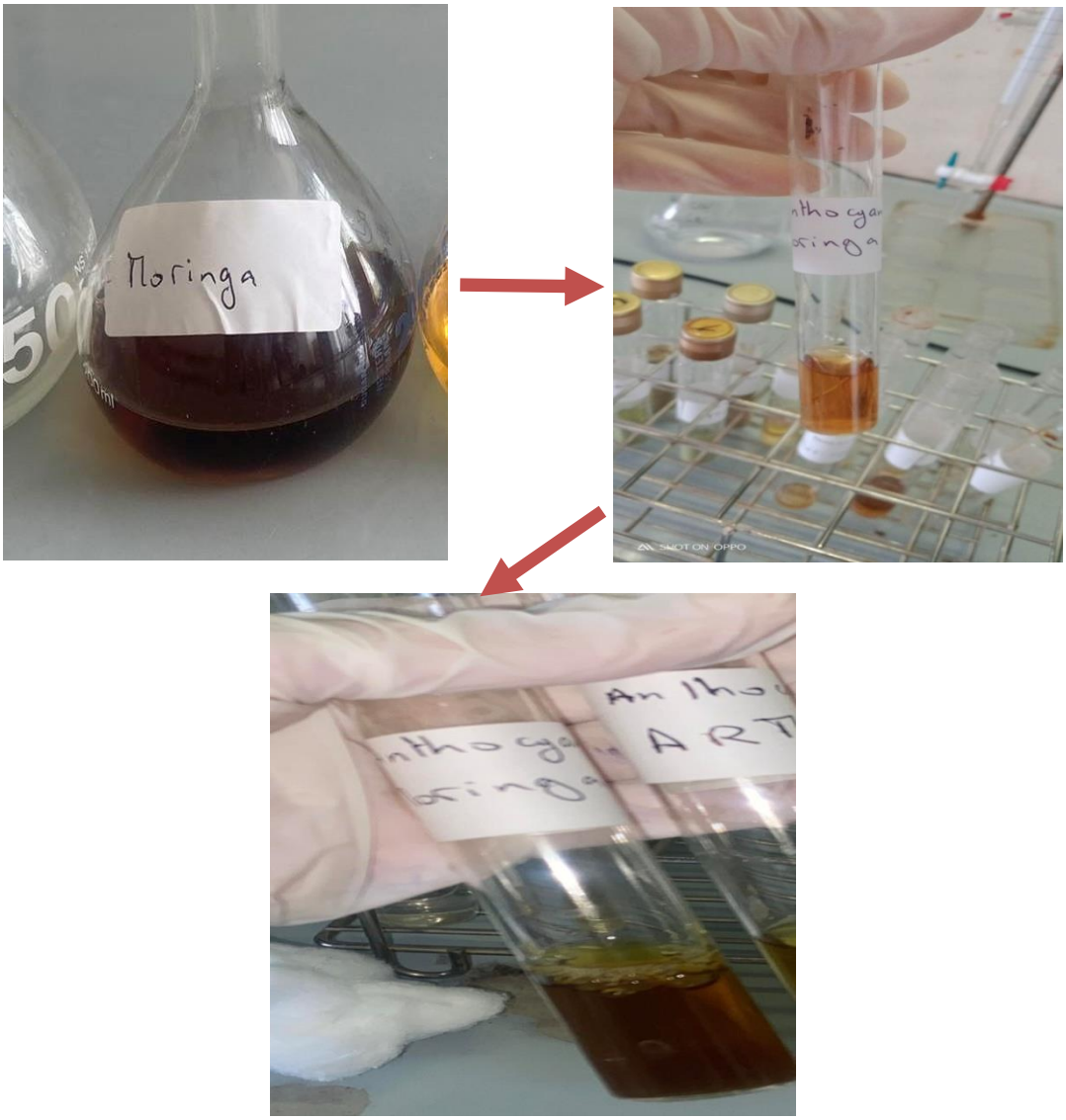


Figure 3.1 : The result of anthocyans in moringa oleifera**(personal photo 2023)**

- ***<u>Aloe vera and Brocchia cinerea :</u>***

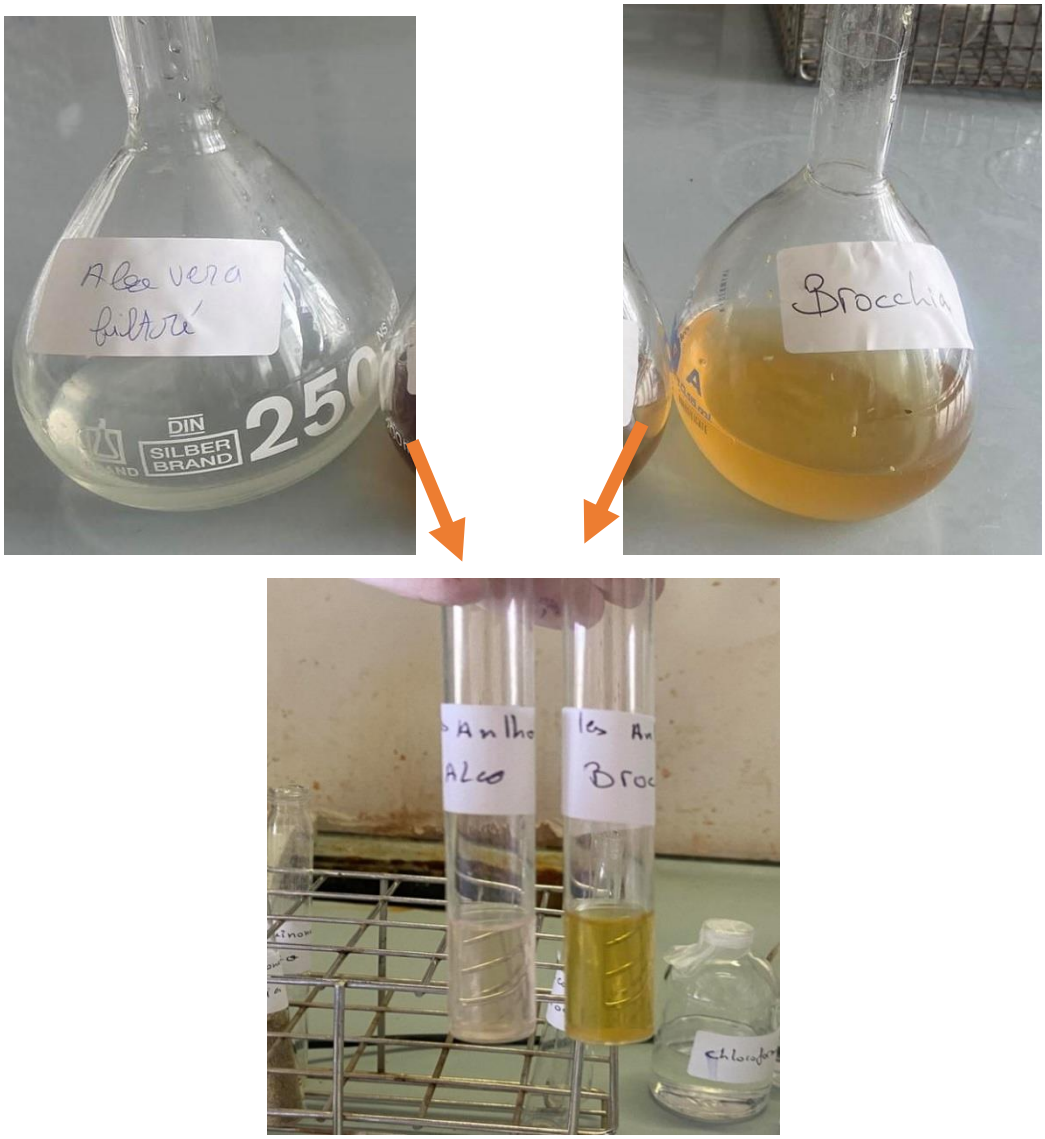


Figure 3.2 : The result of anthocyans in aloe vera and brocchia cenirea plants **(personal photo 2023)**

- ***Artemisia herba alba :***

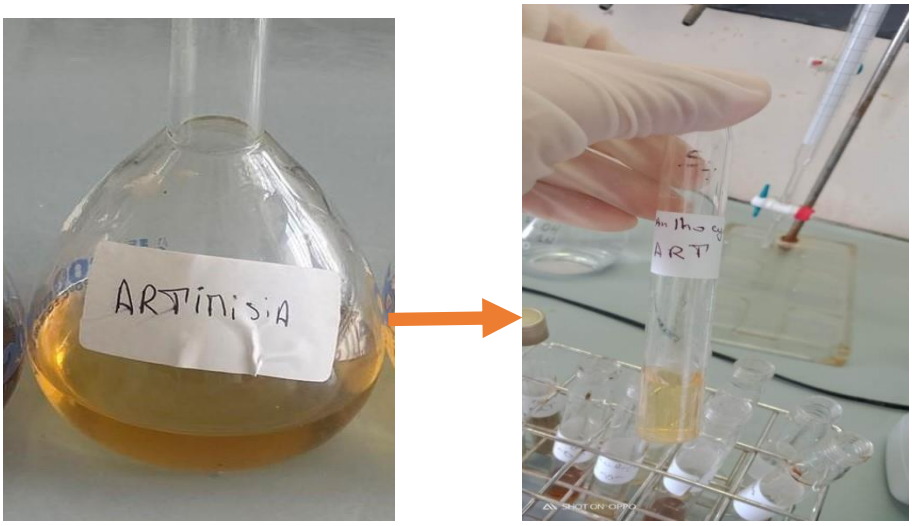


Figure 3.3 : The result of anthocyans in artemisia herba alba **(personal photo 2023)**

✓ **Results:**

The results of this manipulation clearly indicate the absence of anthocyanins characterized by a negative response to the ammonia , this test of anthocyanins was confirmed by the appearance of a red coloration in all the four plants : aloe vera , artemisia herba alba ; brocchia cenirea, moringa oleifera .

➢ **Tanin :**

The presence of tannin in the plant infusion was verified using feCL3 test :

- ***Moringa oleifera :***

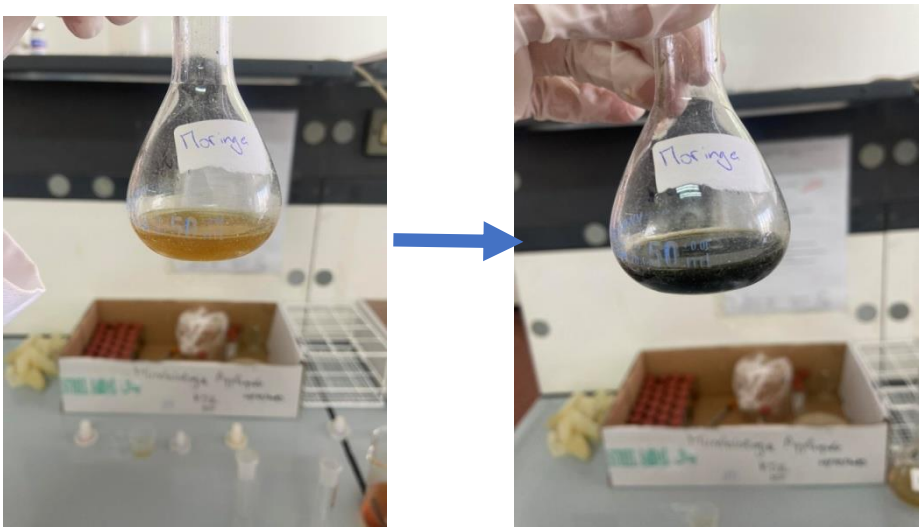


Figure 3.4: The result of tanins in moringa oleifera plant **(personal photo 2023)**

✓ **Result:**

The results of this manipulation clearly indicate the precence of tanins characterized by a positive response to the Fecl3 this test of tanins was confirmed by the appearance of a balack coloration in a moringa plant infused . based on this result *Moringa oleifera* contain tannins

- ***Brocchia cenirea :***

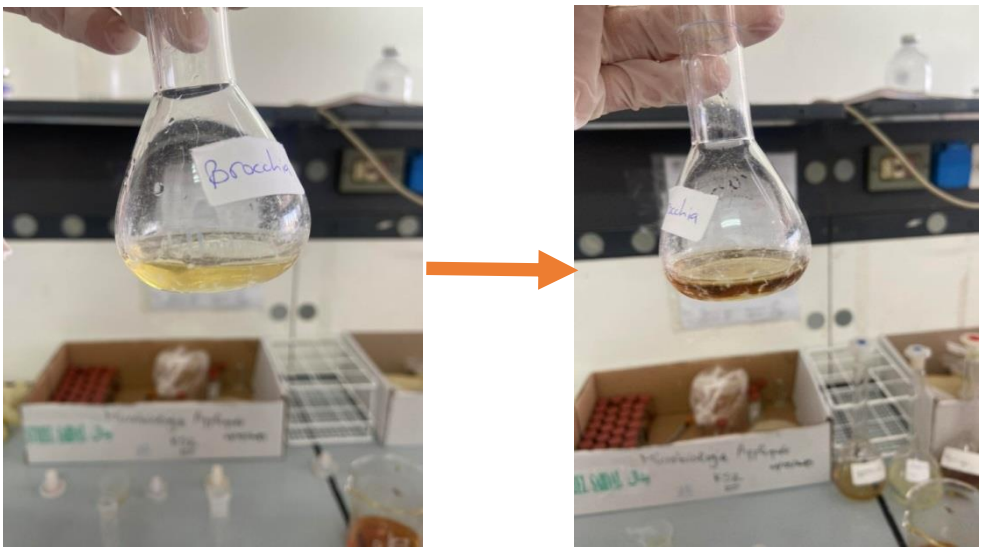


Figure 3.5 : The result of tanin in brocchia cenirea **(personal photo 2023)**

✓ **Result:** The results of this manipulation clearly indicate the precence of tanins characterized by a positive response to the Fecl3 , this test of tanins was confirmed by the appearance of a balack coloration in a *brocchia cenirea* plant infused .

- ***Aloe vera :***

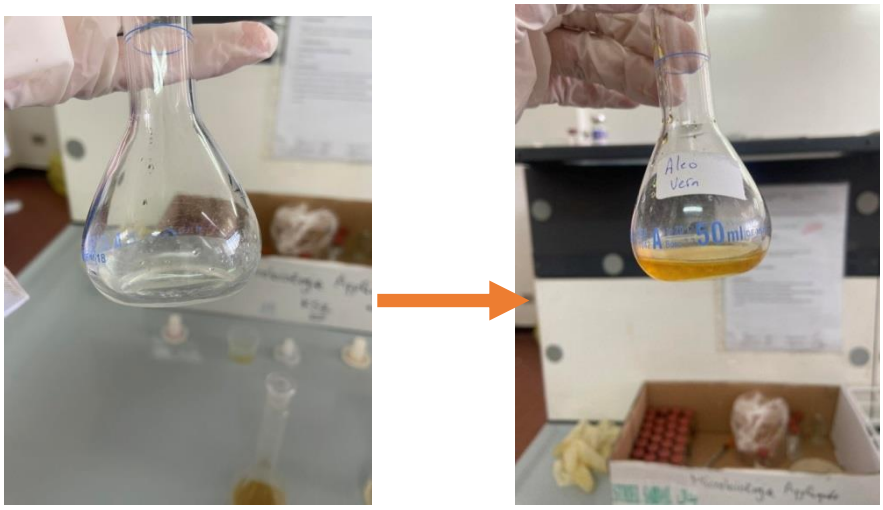


Figure 3.6 : The result of tanins in aloe vera plant **(personal photo 2023)**

✓ **Result:**

The results of this manipulation clearly indicate the presence of tanins characterized by a positive response to the Fecl3 this test of tanins was confirmed by the presence of a balack coloration in aloe vera infused .

- ***Artemisia herba alba :***

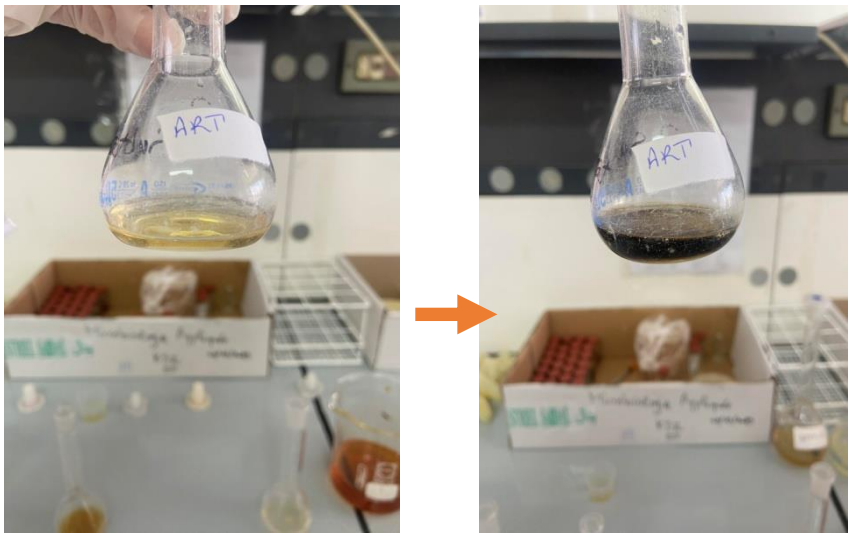


Figure 3.7 : The result of tanins in artemisia herba alba plant **(personal photo 2023)**

✓ **Result :** The results of this manipulation clearly indicate the presence of tanins characterized by a positive response to the Fecl3 this test of tanins was confirmed by the appearance of a balack coloration in Artemisia herba alba infused .

➢ **Mucilage :** The presence of mucilage in the plant infusion was verified using the ethanol test :

- ***Moringa oleifera :***



Figure 3.8 : The result of of mucilage in moringa plant **(personal photo 2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of mucilage characterized by a positive response to the ethanol this test of mucilage was confirmed by the appearance of a precipitation in *moringa* infused

- ***Brocchia cenirea and Aloe vera :***

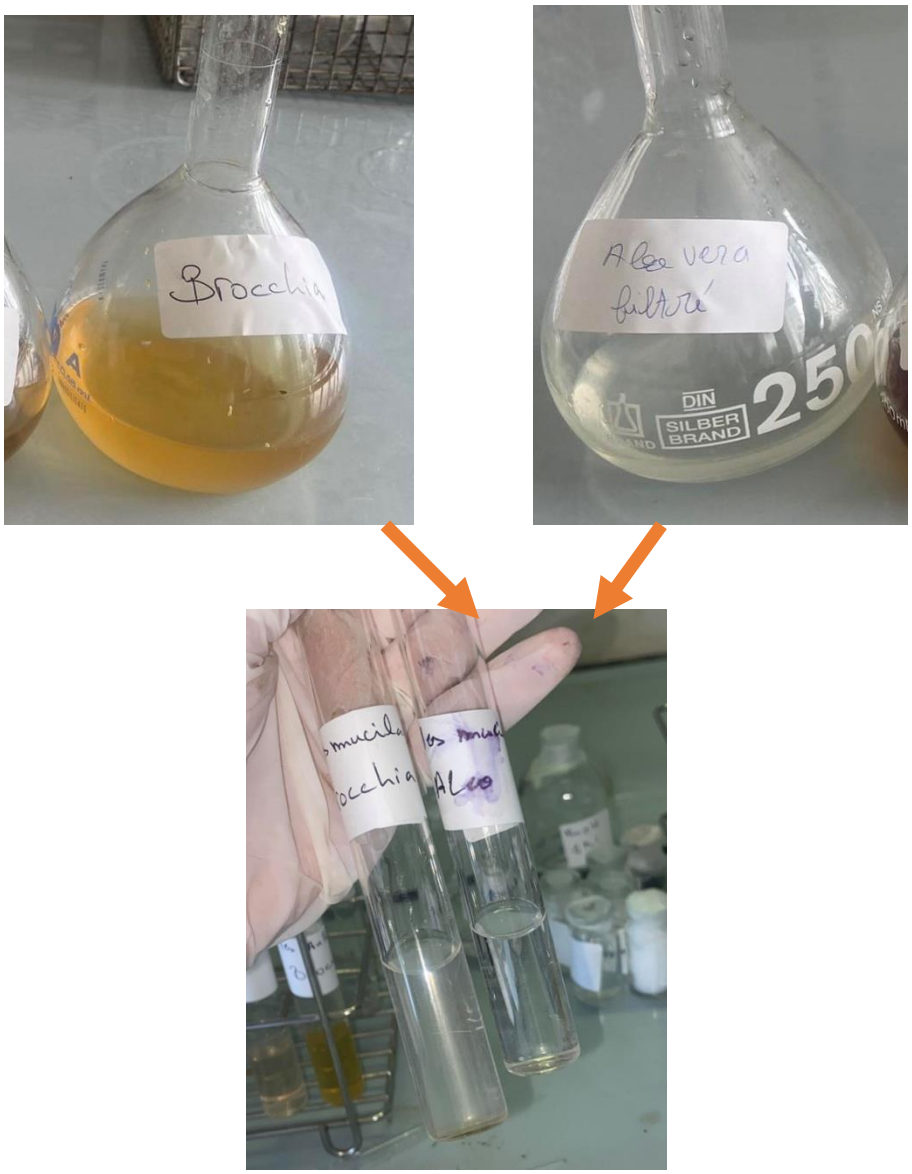


Figure 3.9: The result of mucilage in aloe vera and brocchia cenirea **(personal 2023)**

✓ **Result :** The results of this manipulation clearly indicate the presence of mucilage characterized by a positive response to the ethanol this test of mucilage was confirmed by the appearance of a precipitation in both plants infused .

- ***Artemisia herba alba :***

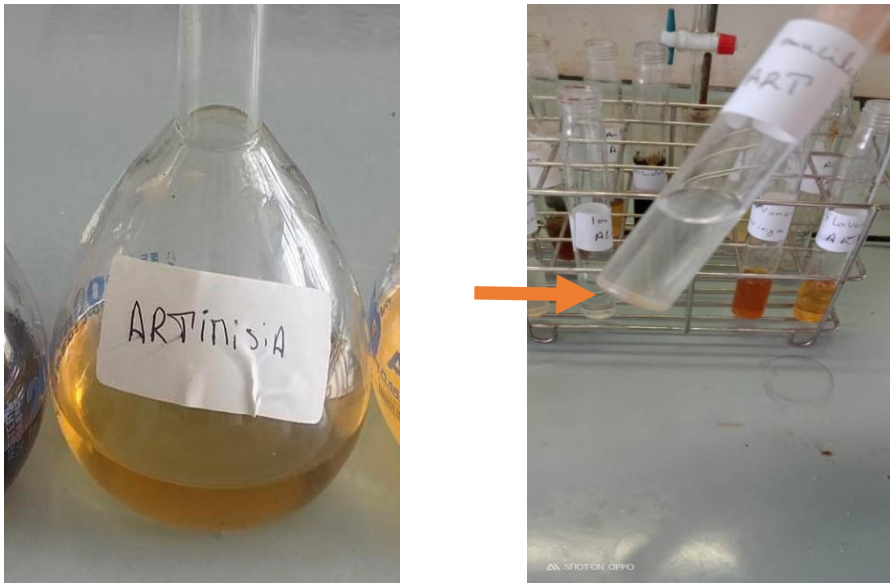


Figure 3.10 : The result of mucilage test in artemisia herba alba **(personal photo)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of mucilage characterized by a positive response to the ethanol this test of mucilage was confirmed by the appearance of a precipitation in artemisia herba alba infused

➢ **Flavinoids :**

The presence of flavonoids in the plant infusion was verified using NAOH test :

- ***Moringa oleifera :***

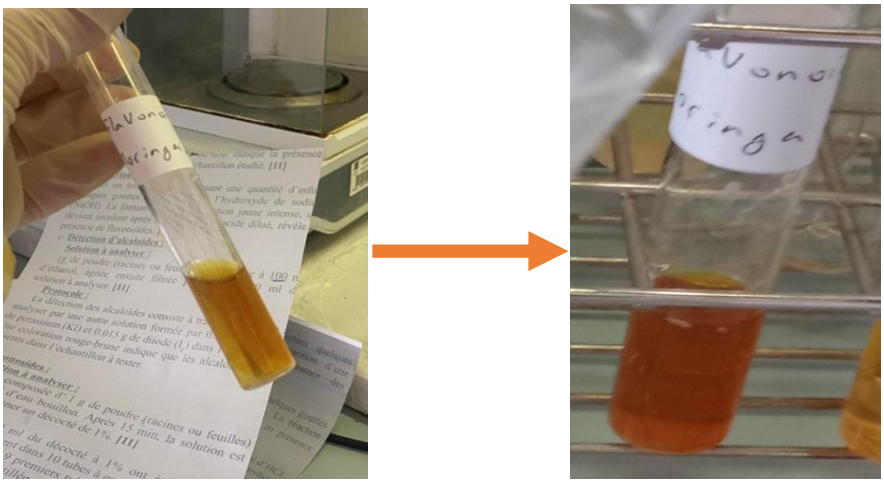


Figure 3.11: The result of flavinoids in moringa oleifera **(personal photo 2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of flavinoids characterized by a positive response to sodium hydroxyde (NAOH) this test of flavinoids was confirmed by the appearance of a yellow color in moringa oleifera infused .

- ***Brocchia cenirea and Aloe vera :***

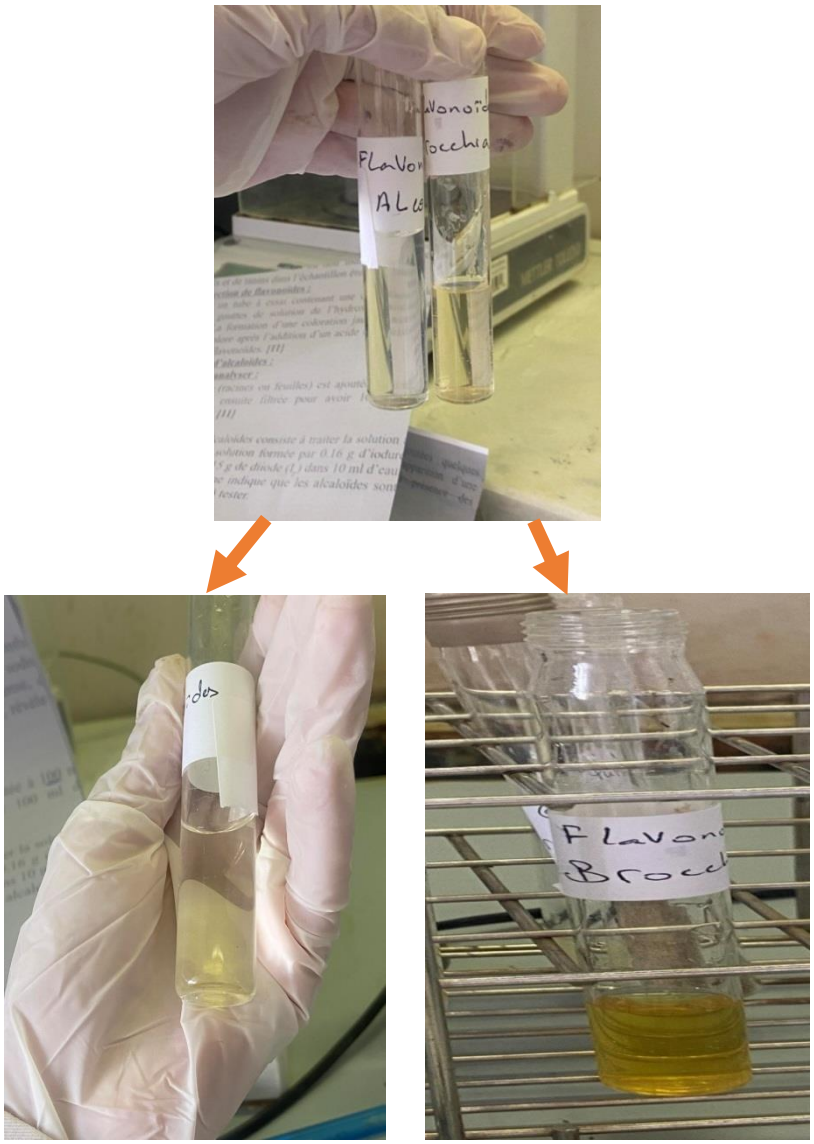


Figure 3.12: The result of falvinoids test in brocchia cenirea and aloe vera **(personal photo 203)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of flavinoids characterized by a positive response to sodium hydroxyde (NAOH) this test of flavinoids was confirmed by the appearance of a yellow color in both plants infused .

- ***Artemisia herba alba :***

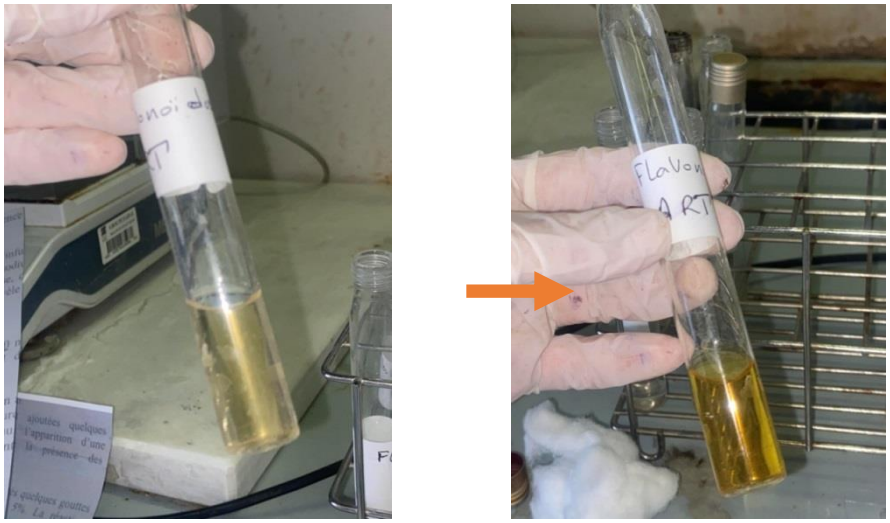


Figure 3.13 : The result of flavinoide test in artemisia herba alba **(personal photo 2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of flavinoids characterized by a positive response to sodium hydroxyde (NAOH) this test of flavinoids was confirmed by the appearance of a yellow color in artemisia herba alba infused .

➢ **Glucosids :**

The presence of Glucosids in the plant powder was verified using sulfuric acide test :

- ***Moringa oleifera and Artemisia herba alba :***

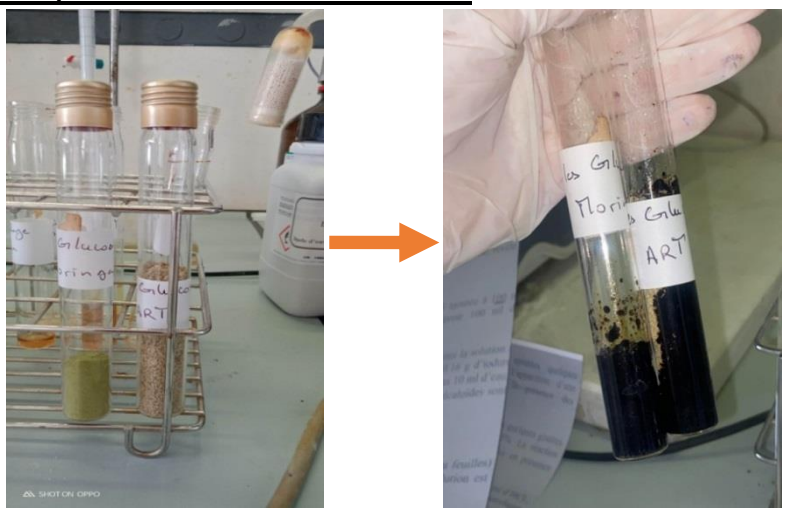


Figure 3.14 : The result of glucosids in moringa oleifera and artemisia herba alba plants **(personal photo 2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of glugosids characterized by a positive response to sulfiruc acide this test of glugosids was confirmed by the appearance of a red color turning to purple color in artemisia herba alba and moringa oleifera powder .

- ***Brocchia cenirea and Aloe vera :***

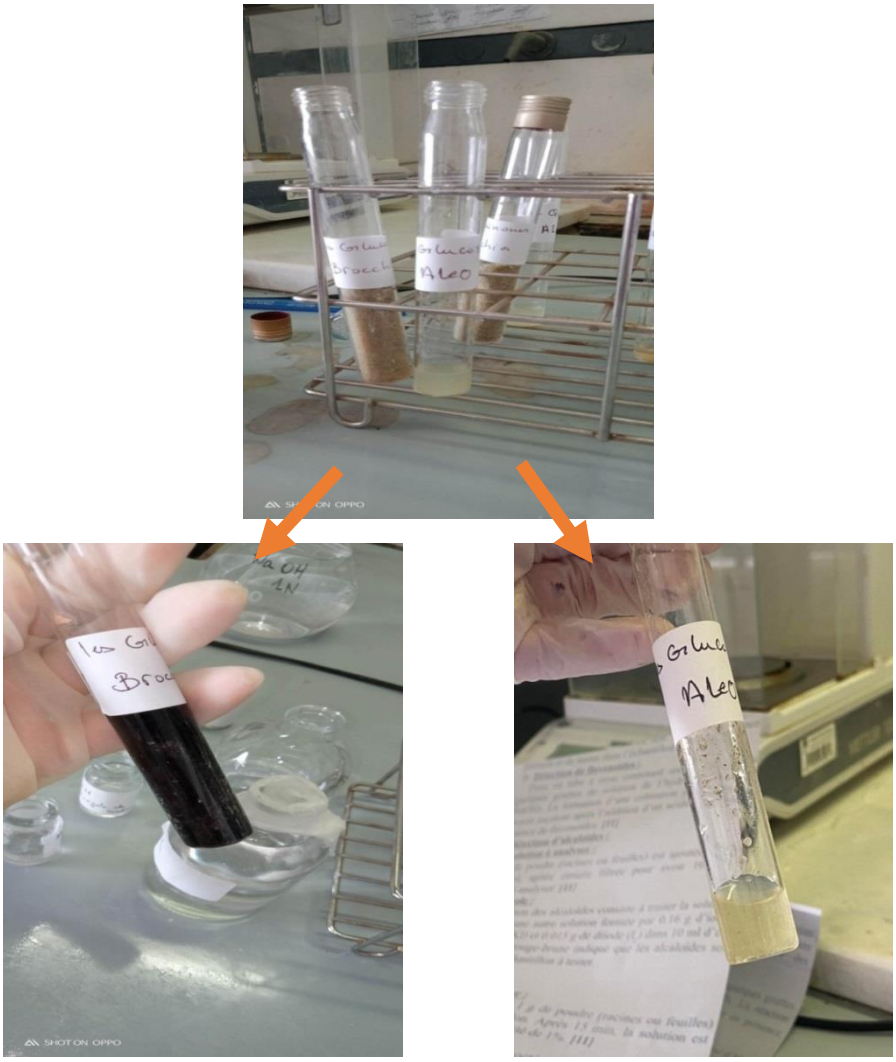


Figure 3.15 : The result of glucosids test in aloe vera gel and brocchia cenirea powder **(personal photo 2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of glugosids characterized by a positive response to sulfiruc acide this test of glugosids was confirmed by the appearance of a red color turning to purple color in brocchia cenirea powder and aloe vera gel

➢ **Quinons:**

The presence of Quinons in the plant powder was verified using HCL and chloroforme test :

- ***Moringa oleifera and artemisia herba alba and brocchia cenirea and aloe vera :***

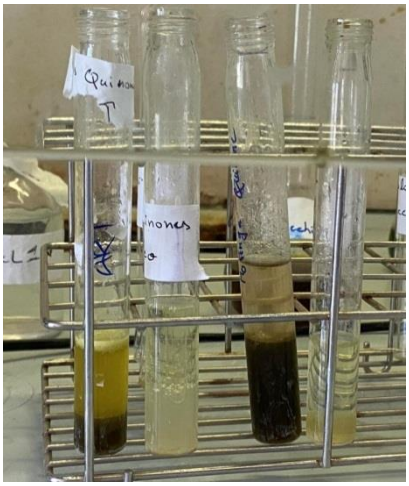


Figure 3.16 : The results of quinons in artemisia herba alba and moringa oleifera **(personal photo2023)**

✓ **Result :**

The results of this manipulation clearly indicate the presence of Quinons characterized by a negative response to vhloroforme this test of glugosids was confirmed by the absence of a red color in four plants.

➢ **Alkaloids :**

The presence of alkaloids in the plant powder was verified using ethanol and KI and I2 test :

- ***Moringa oleifera* :**

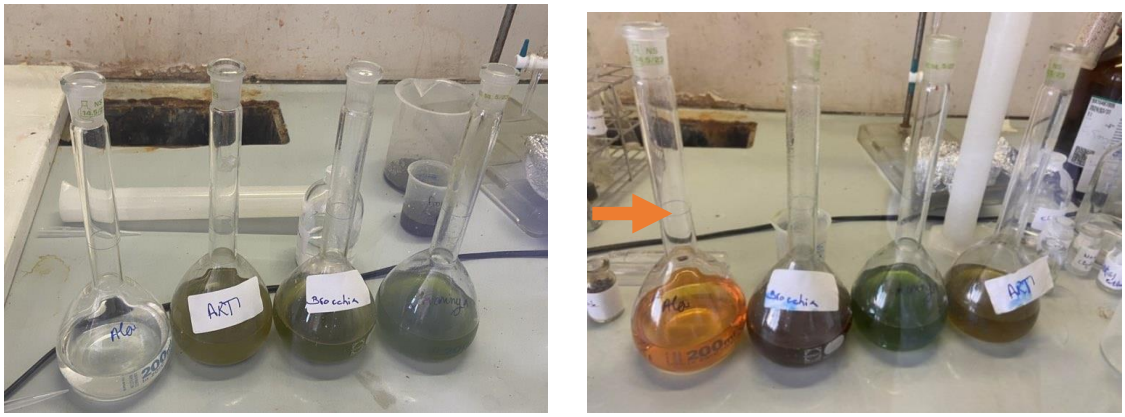
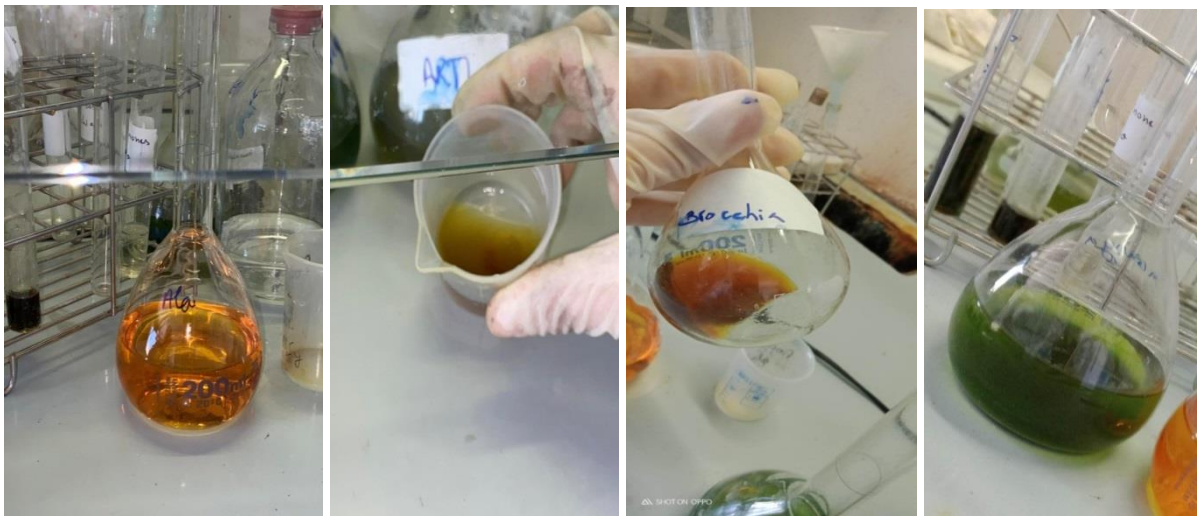


Figure 3.17 : The result of phytochemical screening of alkaloids in four plants **(personal photo 2023)**



Aloe vera **artemisia herba alb** **Brocchia cenirea** **moringa oleifera**

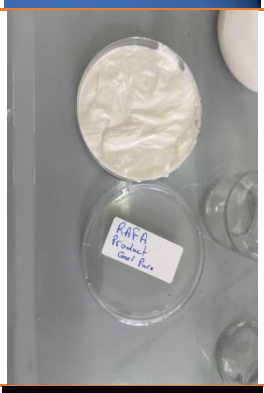
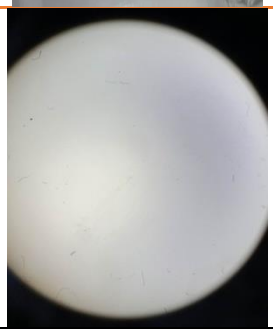
Figure 3.18: The result of phytochemical screening of alkaloids in the four plant **(personal photo)**

3.3. The results of the creams :

The study involved the preparation of two different aloe vera creams using almond oil as a base. The pH value of the first cream(pure gel) was measured to be 5.12 and the second cream(active ingredient) was 6.12 The color of the creams was found to be white.the test of lumps was conducted to check whether there were any lumps in the cream or no The microscopic analysis of the results revealed that the absence of these lumps is indicative of a favorable textural quality.

Table 3.3 : The results of PH and color and texture of both creams

The tests	The results
PH(the second cream) (active ingredients)	

PH(the second cream) (active ingredients)	
Color	
Lumps (texture)under the microscope	

3.4. The Evaluation of antimicrobial activity:

3.4.1.Plants extracts:

Antibacterial or antifungal activity was determined by measuring the diameter of the zone of inhibition, represented by a halo formed around each disc where no growth was observed.

The antibacterial spectrum, antifungal spectrum and classification of bacterial and fungal strains in the extract are as follows:

-Diameter less than 8mm: not sensitive (-) or resistant.

-Diameter between 9mm to 14mm: sensitive (+).

-Diameter between 15mm to 19mm: highly sensitive (++).

-Diameter greater than 20mm: extremely sensitive (+++).

3.5. Antibiogram:

The contact of different strains with antibiotics allowed for the realization of an antibiogram to determine the diameter of inhibition and consequently assess bacterial sensitivity or

resistance towards them. We tested the activity of the antibiotic Amoxicillin, aztreonam and streptomycin on sixsixfive bacterial strains and Nystatin on three fungal strains using the standard disk method. The measurements of the inhibition zones are presented in tables and their appearances are photographed in Figure.

Table 3.4: Diameter of inhibition zones in mm and sensitivity of microbial strains to antibiotics.

Strains *ATB*		*E. coli*	*B. subtilus*	*P. aeruginosa*	*S. aureus*	*Salmonella typhimurium*
Amoxicillin	**Diameter**	23.4	34.2	12.2	50.1	31.8
	Sensitivity	+++++	+++++	+	+++++	+++++
aztreonam	**Diameter**	23.3	14.0	11.7	0	21.0
	Sensitivity	+++++	+	+	-	+++++
streptomycin	**Diameter**	27.8	32.4	25.7	24.6	24.2
	Sensitivity	+++++	+++++	+++++	+++++	+++

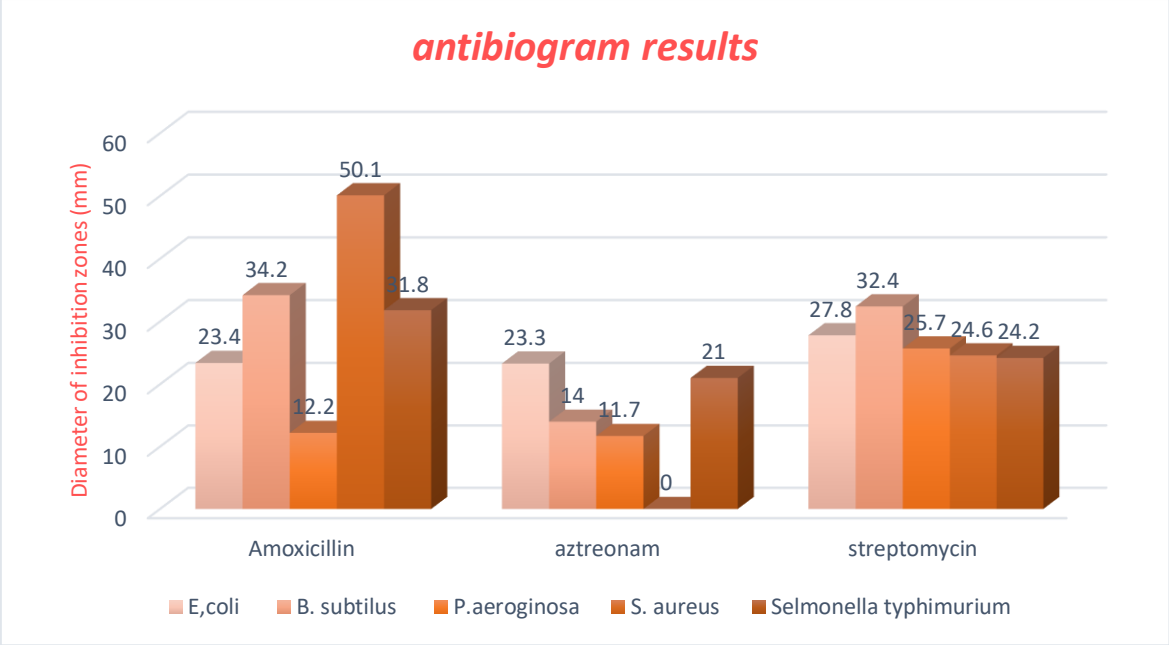


Figure 3.19: Antibiotic activity against the strain tested

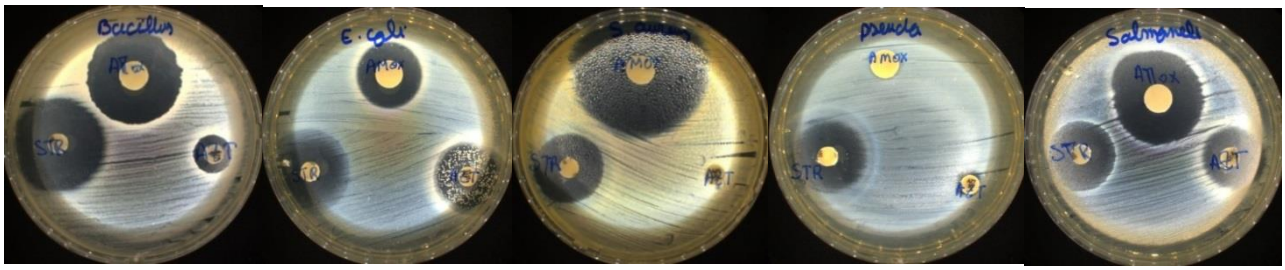


Figure 3.20: Appearance of antibiotic inhibition zones with respect to bacterial strains(**personal photos)**

✓ ***Results:***

The mentioned results show that all strains were sensitive to all antibiotics, except S. aureus, which was resistant to aztreonam.

S. aureus was the most sensitive strain to amoxicillin with an inhibition zone of 50.1 mm, followed by B. subtilis, Salmonella typhimurium, E. coli, and P. aeruginosa.

E. coli was the most sensitive strain to aztreonam with an inhibition zone of 23.3 mm, followed by Salmonella typhimurium,B. subtilis, and P. aeruginosa, S. aureus was resistant to this antibiotic.

B. subtilis was the most sensitive strain to streptomycin with an inhibition zone of 32.4 mm, followed by E. coli,P. aeruginosa,S. aureus, and Salmonella typhimurium.

3.6. The Evaluation of the antibacterial activity of the extracts and essential oils :

Plants contain numerous compounds endowed with antimicrobial activity.. The antimicrobial potency of plant extracts depends on their chemical compositions.

Our study focused on evaluating the antibacterial effect and antifungal of different extracts and essential oils from moringa oleifera , brocchia cenirea , Artemisia herba alba , aloe vera . The sensitivity test was performed using the disc diffusion method (aromatogramme), and the diameters of the inhibition zones were measured by colony counter .

3.6.1 The antibacterial effect of the plant extracts:

The antibacterial effect of the studied plant extracts (methanolic /aqueose/hydroeyhanolic) is evaluated in this study using the agar diffusion technique. The results obtained are represented in the following figures and tables.

3.6.1.1Moringa oleifera:

- **Methanolic extract:**

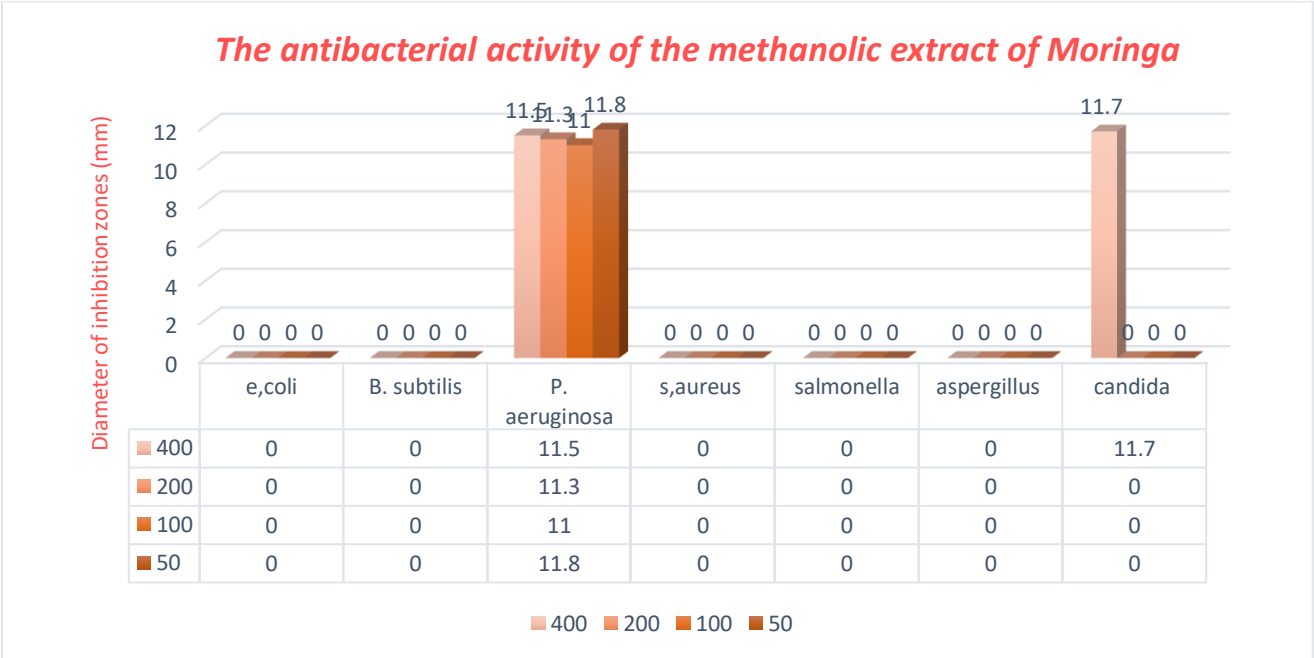


Figure 3.21: Antibacterial activity of the methanolic extract of Moringa oleifera expressed by zone diameters in mm

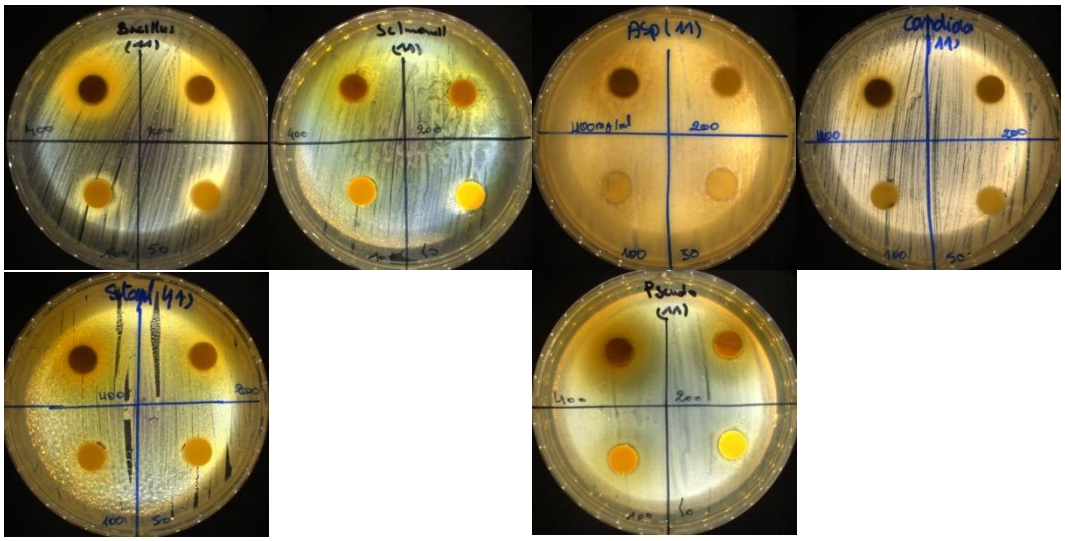


Figure 3.22 : Results of the antibacterial effect of the methanolic extractof *Moringa oleifera* **(personal photo,2023).**

- ✓ ***Results:***

we have observed from the results obtained in our study that the methanolic extract of moringa oleifera demonstrates antimicrobial activity against certain bacterial strains.

The results of our study, based on the diameters of the inhibition zones, allow us to interpret that:

- For ***E. coli/ B. subtilis /S. aureus /salmonella/aspergillus***:No inhibition zones were observed for all concentrations of methanolic extract of Moringa oleifera (400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml), which means that are resistant to this extract.
- ***P. aeruginosa***: The inhibition zone diameters are 11.5 mm, 11.3 mm, 11 mm, and 11.8 mm for concentrations of 400 mg/ml, 200 mg/ml et 100 mg/ml, and 50 mg/ml, respectively, indicating that P. aeruginosa is sensitive to the methanolic extract of moringa oleifera
- ***Candida albicans***: A diameter of 11.7 mm inhibition zone was observed for the concentration of 400 mg/ml, indicating that Candida albicans is sensitive to this extract. No inhibition zones were observed for the other extract concentrationswhitch mean that is resistance to the extract.

- **Aqueous extract:**

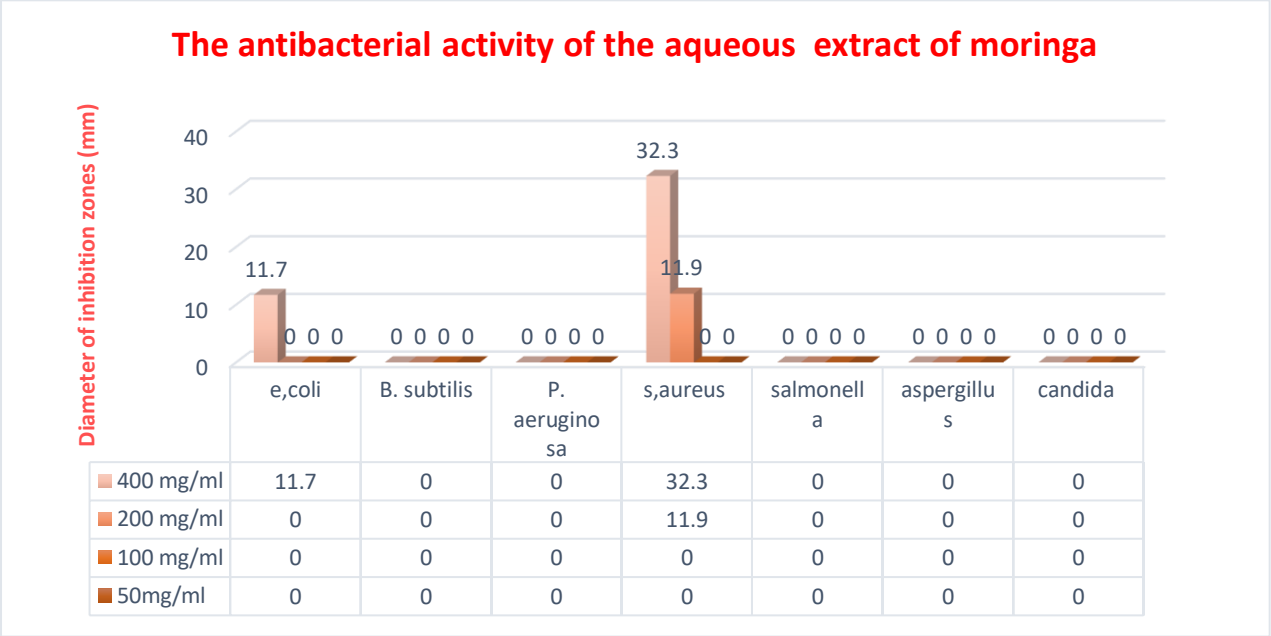


Figure 3.23: Antibacterial activity of the aqueous extract of Moringa oleifera expressed by zone diameters in mm

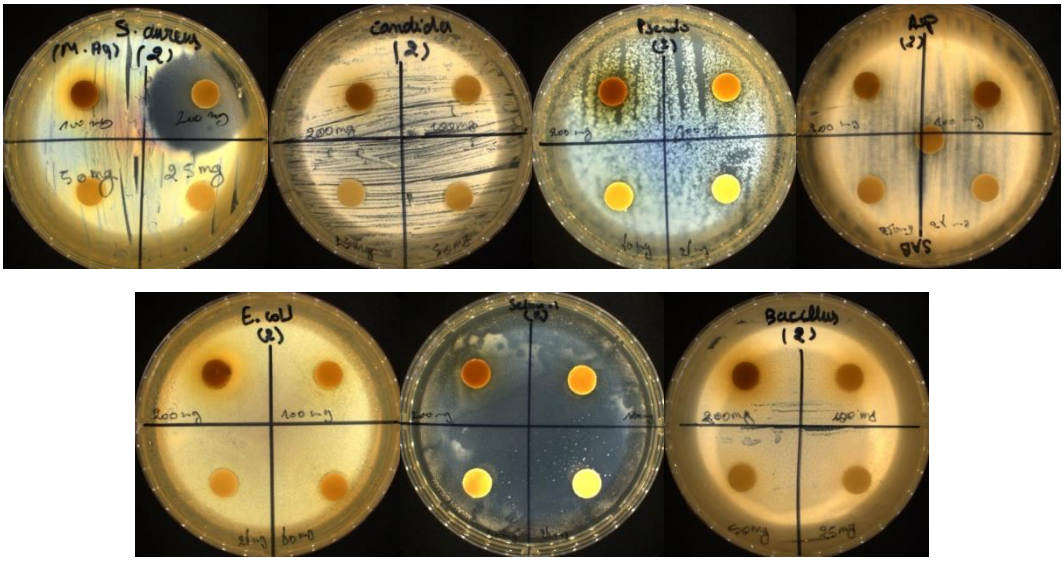


Figure 3.24: Results of the antibacterial effect of the aqueous extract of Moringa oleifera **(personal photos).**

✓ **Results:**

The results of the antimicrobial effect of the aqueous extract of Moringa are interpreted as follows:

- ***E. coli:***A diameter of 11.7 mm for the inhibition zone was observed at the concentration of 400 mg/ml, suggesting that candida albicans is sensitive to this extract. Conversely, no inhibition zones were observed for the remaining extract concentrations (200 mg/ml,100 mg/ml,50 mg/ml), implying resistance to the extract.

- ***<u>For B. subtilis/P. Aeruginosa /salmonella/ aspergillus/ candida albicans:</u>***

A diameter of 0 mm was observed for all concentrations, indicating that the aqueous extract of moringa oleifera did not inhibit the growth of this strains at any of the tested concentrations. making it resistance to this extract.

- **<u>Hydroethanolic extract:</u>**

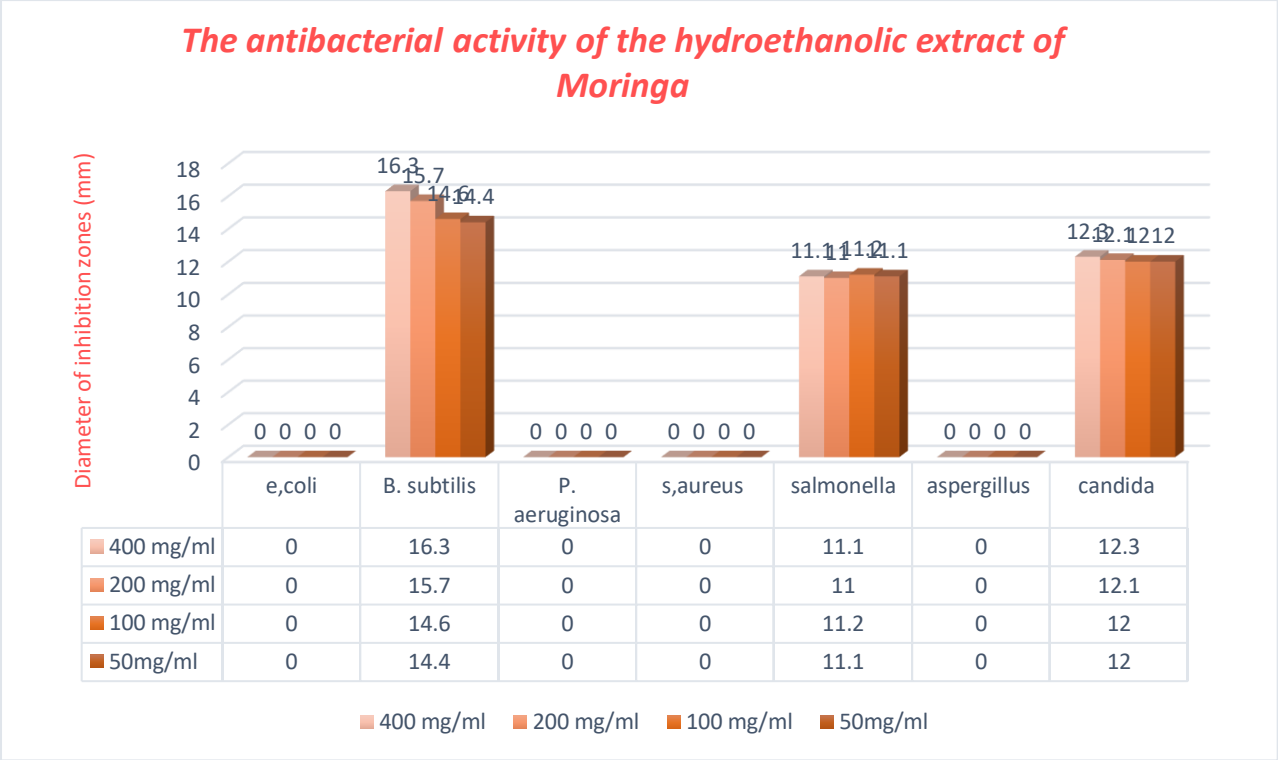


Figure 3.25: Antibacterial activity of the hydroethanolic extract of Moringa oleifera expressed by zone diameters in ***mm***

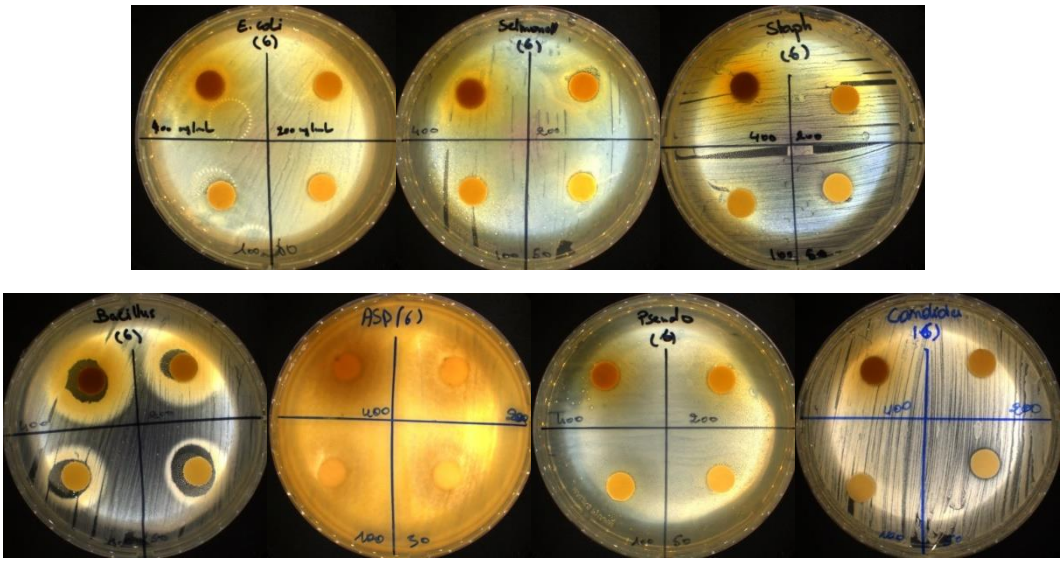


Figure 3.26: Results of the antibacterial effect of the hydroethanolic extract of Moringa oleifera (personal photos).

✓ **Results:**

The hydroethanolic extract of moringa oleifera did not show any inhibitory effects on the growth of E. coli, P. aeruginosa, S. aureus, and Aspergillus at any of the tested concentrationsthisresult suggests thatthese strains are resistance to the hydroethanolic extract of moringa. On the other hand:

- ***Candida albicans***:The inhibition zone diameters are 16.3 mm, 15.7 mm, 14.6 mm, and 14.4 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that the substances exhibited inhibitory effects against B. subtilis, with the largest inhibition observed at a concentration of 400 mg/ml (16.3mm) then 15.7 mm for concentration of 200 mg/ml. that mean b. subtilisis very sensitive to this concentration,and sensitive to the to last concentration (100 mg/ml,50 mg/ml).
- ***Salmonella***: The inhibition zone diameters are 11.1 mm, 11 mm, 11.2 mm, and 11.1 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values suggest that salmonella sensitive to the hydroethanolic extract of moringa oleifera, with the largest inhibition (11.2 mm) observed at a concentration of 100 mg/ml.
- ***Candida albicans***:The inhibition zone diameters are 12.3 mm, 12.1 mm, 12 mm, and 12 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that the hydroethanolic extract of moringa oleifera exhibited inhibitory effects against Candida, that mean candida albicans is sensitive to this extract with the largest inhibition (12.3mm) observed at a concentration of 400 mg/ml.

3.6.1.2. Brocchia cinerea:

- **.Methanolic extract:**

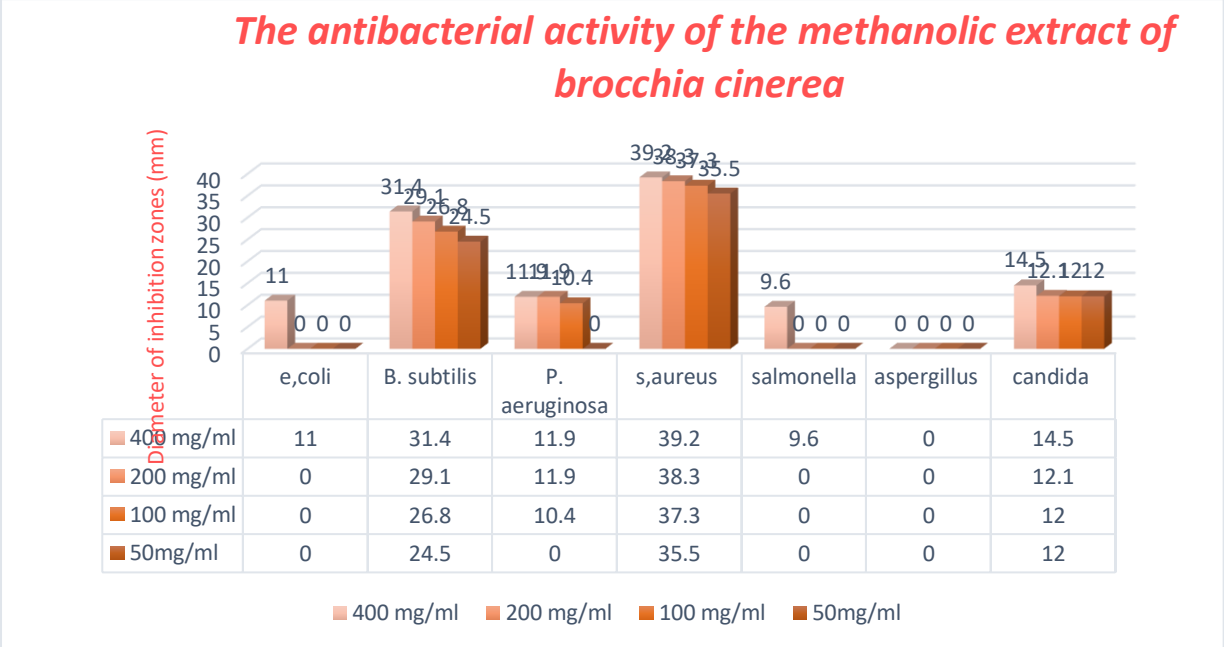


Figure 3.27: Antibacterial activity of the methanolic extract of Brocchia cinerea expressed by zone diameters in mm

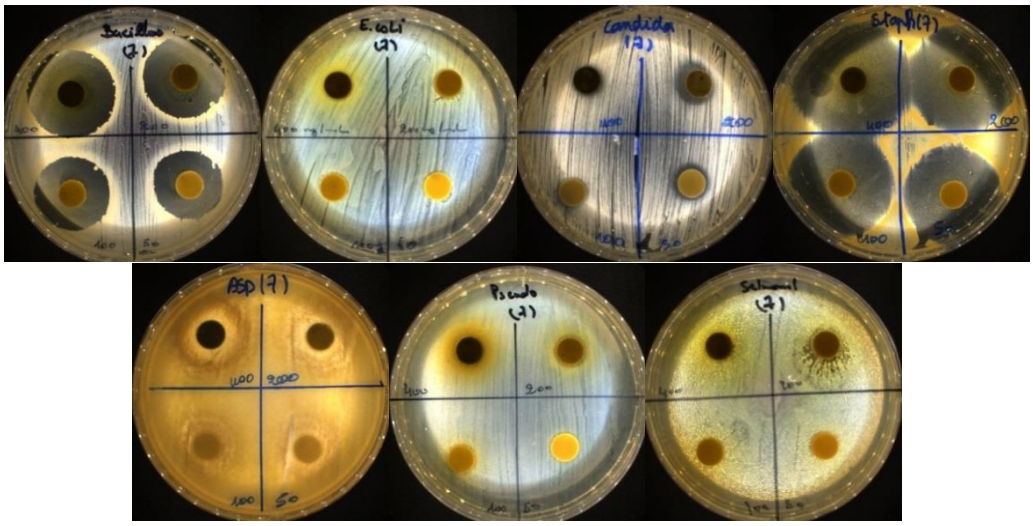


Figure 3.28: Results of the antibacterial effect of the methanolic extract of *brocchia cinerea* **(personal photos).**

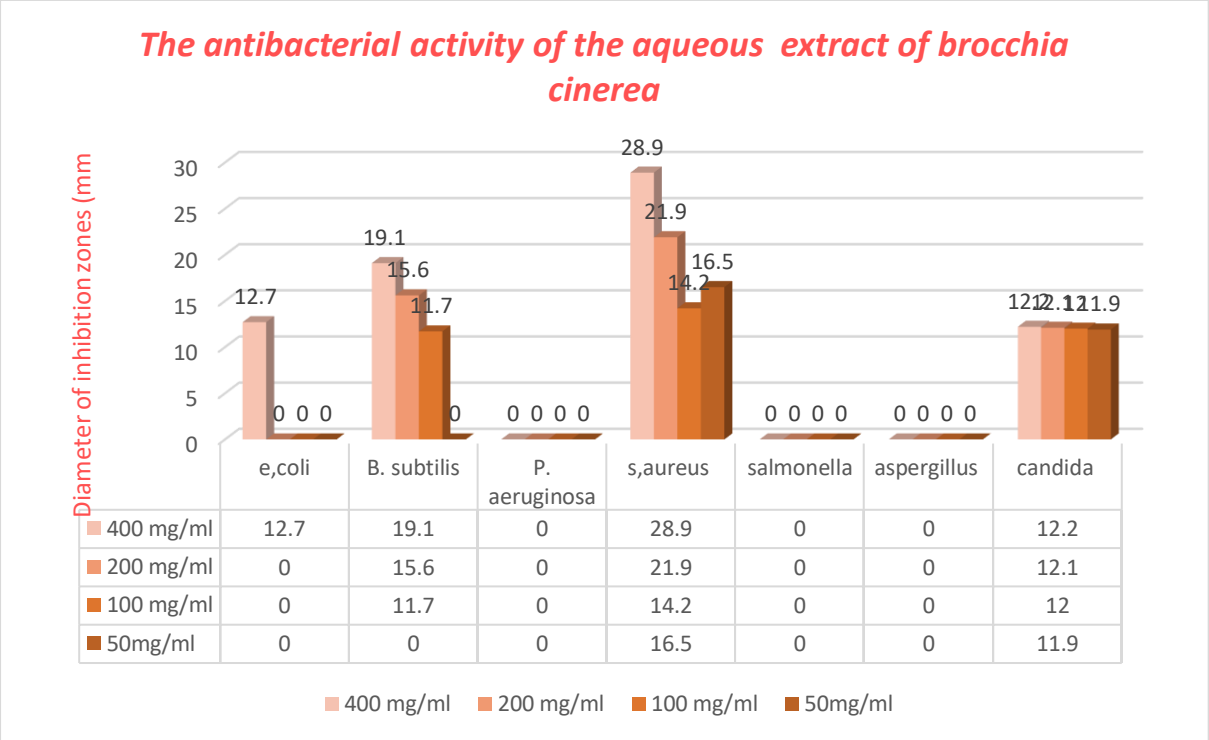
✓ **Results:**

In order to assess the antimicrobial effectiveness of the methanolic extract of Brocchia cinerea, the diameters of the inhibition zones were measured. The interpretation of the results regarding the antimicrobial effect of the substances is as follows:

- E. coli: The inhibition zone diameters are 11 mm, 0 mm, 0 mm, and 0 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that the methanolic extract of brocchia cinerea exhibited inhibitory effects against E. coli, with the largest inhibition observed at a concentration of 400 mg/ml. E. coli is sensitive to the extract at this concentration.
- B. subtilis: The inhibition zone diameters are 31.4 mm, 29.1 mm, 26.8 mm, and 24.5 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that the substances showed inhibitory effects against B. subtilis, with the largest inhibition observed at a concentration of 400 mg/ml. B. subtilis is highly sensitive to the methanolic extract of brocchia cinerea
- P. aeruginosa: The inhibition zone diameters are 11.9 mm, 11.9 mm, 10.4 mm, and 0 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values suggest thatthe methanolic extract of brocchia cinerea exhibited inhibitory effects against P. aeruginosa, with the highest inhibition observed at concentrations of 400 mg/ml and 200 mg/ml. that mean P. aeruginosa is sensitive to this extract at a concentration of 400 mg/ml,200 mg/ml,100 mg/ml and resistant to the concentration of 50 mg/ml.
- S. aureus: The inhibition zone diameters are 39.2 mm, 38.3 mm, 37.3 mm, and 35.5 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that the methanolic extract of brocchia cinerea showed inhibitory effects against S. aureus, with the largest inhibition observed at a concentration of 400 mg/ml. S. aureus is highly sensitive to the methanolic extract of brocchia cinerea.
- Salmonella: The inhibition zone diameters are 9.6 mm, 0 mm, 0 mm, and 0 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that salmonella is sensitive to the methanolic extract of brocchia cinerea at a concentration of 200 mg/ml.

- Aspergillus: 0 mm for all concentrations. Aspergillus is resistant to the methanolic extract of brocchia cinerea
- Candida albicans: The inhibition zone diameters are 14.5 mm, 12.1 mm, 12 mm, and 12 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. These values indicate that Candida albicans is sensitive to the methanolic extract of brocchia cinerea, with the highest inhibition observed at a concentration of 200 mg/ml

- **.Aqueous extract:**



	e,coli	B. subtilis	P. aeruginosa	s,aureus	salmonella	aspergillus	candida
400 mg/ml	12.7	19.1	0	28.9	0	0	12.2
200 mg/ml	0	15.6	0	21.9	0	0	12.1
100 mg/ml	0	11.7	0	14.2	0	0	12
50mg/ml	0	0	0	16.5	0	0	11.9

Figure 3.29: Antibacterial activity of the aqueous extract of Brocchia cinerea expressed by zone diameters in mm

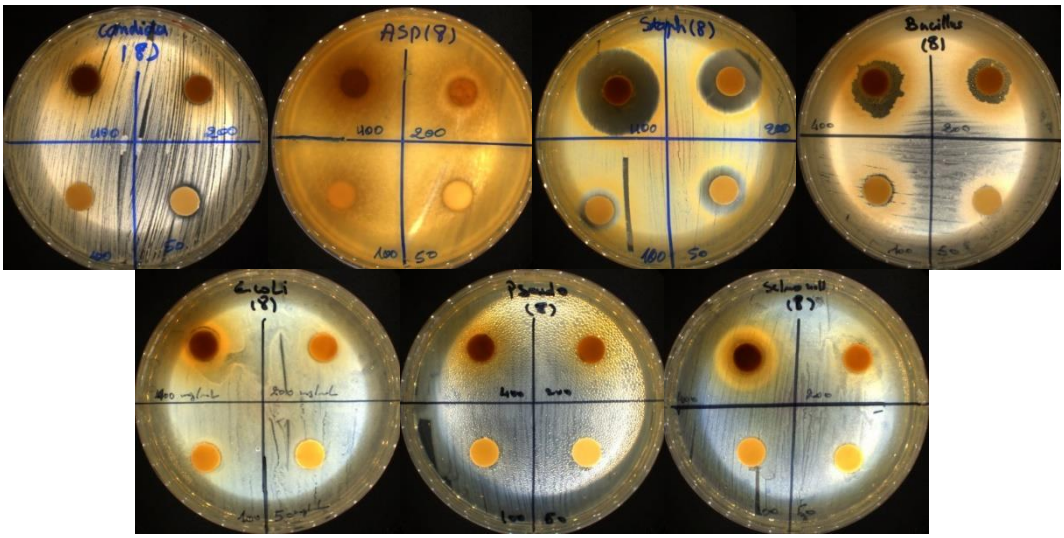


Figure 3.30: Results of the antibacterial effect of the aqueous extract of *brocchia cinerea* (personal photos).

✓ **Results:**

The aqueous extract of brocchia cinerea exhibited variable inhibitory effects against the different strains. They were effective against E. coli (at 400 mg/ml), B. subtilis (at all concentrations), S. aureus (at all concentrations), and Candida (at all concentrations). However, no inhibitory effects were observed against P. aeruginosa, Salmonella, and Aspergillus.

- P. aeruginosa, Salmonella, and Aspergillus: A diameter of 0 mm was observed for all concentrations, indicating that the aqueous extract of brocchia cinerea did not inhibit the growth of this strains at any of the tested concentrations. making it resistance to this extract.
- E. coli: The extract displayed inhibitory activity against E. coli, with the highest observed inhibition zone diameter of 12.7 mm at a concentration of 400 mg/ml. However, no inhibitory effects were observed at lower concentrations (200 mg/ml, 100 mg/ml, and 50 mg/ml). E. coli is sensitive to the aqueous extract of brocchia cinerea.
- B. subtilis: The extract exhibited inhibitory effects on B. subtilis growth, with decreasing inhibition zone diameters as the concentration decreased. The largest inhibition zone diameter of 19.1 mm was observed at a concentration of 400 mg/ml, followed by 15.6 mm at 200 mg/ml, 11.7 mm at 100 mg/ml, and no inhibition at 50 mg/ml.b. subtilis is sensitive to the aqueous extract of brocchia cinerea at a concentration of 100 mg/ml and very sensitive at the concentration of 400 mg/ml and 200 mg/ml.
- S. aureus: The aqueous extract of brocchia cinerea demonstrated inhibitory effects on S. aureus, with decreasing inhibition zone diameters as the concentration decreased. The largest inhibition zone diameter of 28.9 mm was observed at a concentration of 400 mg/ml, followed by 21.9 mm at 200 mg/ml, 14.2 mm at 100 mg/ml, and 16.5 mm at 50 mg/ml. S aureus is extremely sensitive to the aqueous extract of brocchia cinerea at the concentration of 400 mg/ml and 200 mg/ml, and very sensitive at the concentration of 100 mg/ml and 50 mg/ml
- Candida albicans: The aqueous extract of brocchia cinerea displayed inhibitory effects on Candida growth, with similar inhibition zone diameters observed at all concentrations. The inhibition zone diameters were 12.2 mm, 12.1 mm, 12 mm, and 11.9mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. Candida albicans is sensitive to the extract.

- **Hydroethanolic extract:**

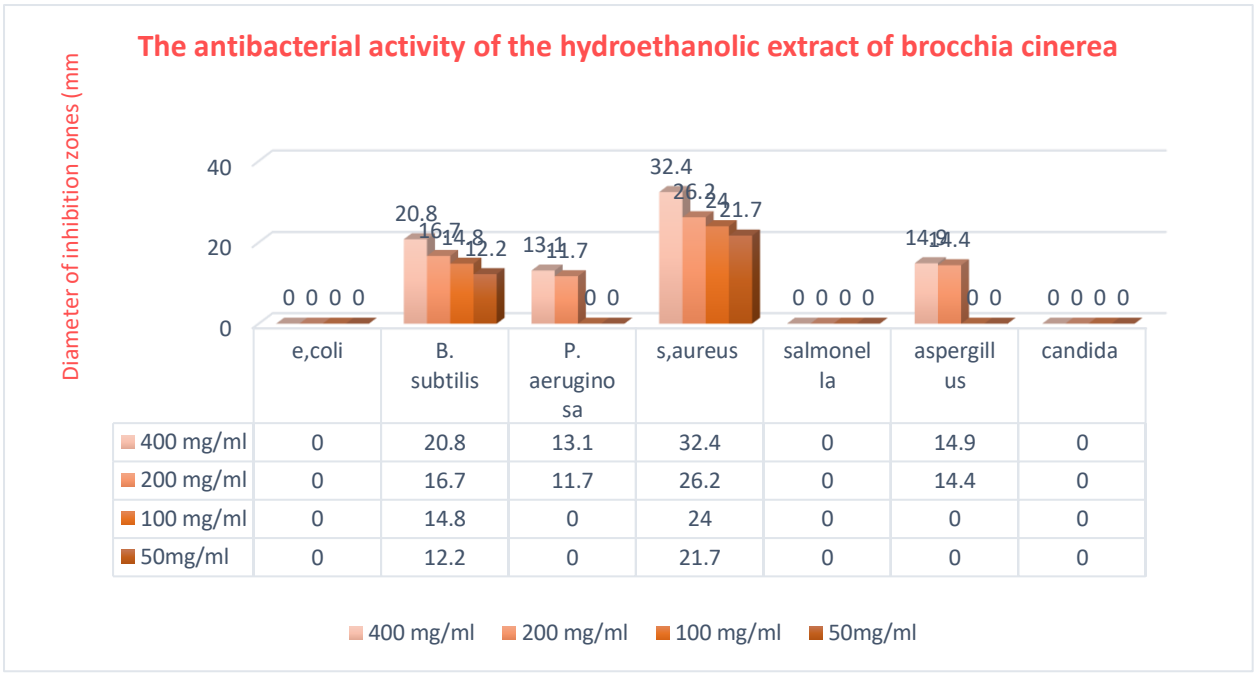


Figure3.31 : Antibacterial activity of the hydroethanolic extract of Brocchia cinerea expressed by zone diameters in mm

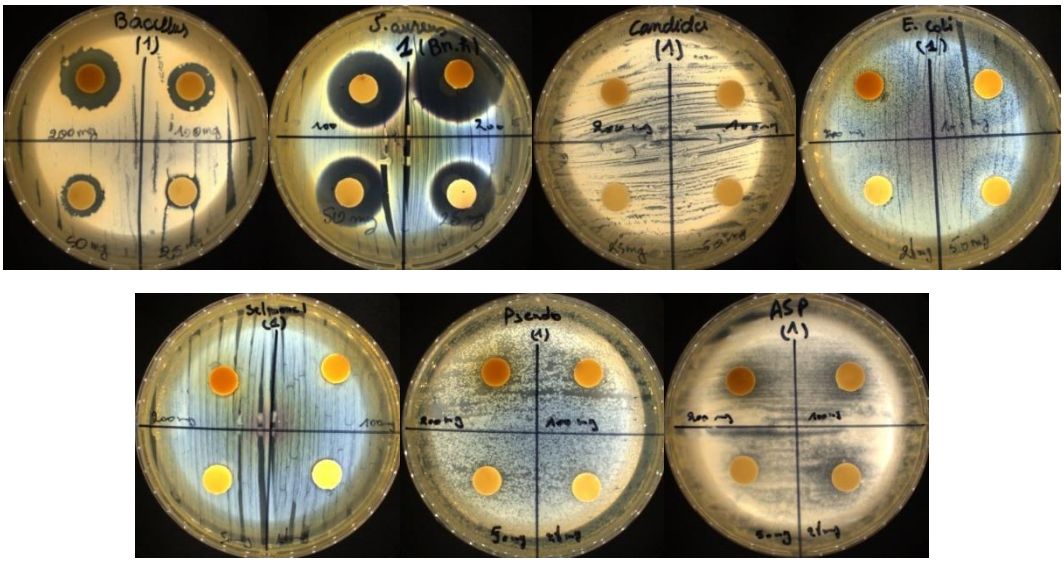


Figure3.32: Results of the antibacterial effect of the hydroethanolic extract of *brocchia cinerea* **(personal photos).**

✓ **Results:**

The hydroethanolic extract of brocchia cinerea showed variable inhibitory effects against the tested strains. They were effective against B. subtilis, P. aeruginosa (at higher concentrations 400 mg/ml and 200 mg/ml), S. aureus, and Aspergillus. No inhibitory effects were observed against E. coli, Salmonella, and Candida

- For E. coli /salmonella / candida albicans: No inhibitory effects of the hydroethanolic extract of Brocchia cinerea were observed against E. coli at any of the tested concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml). this strain are resistant to the extract

- B. subtilis:The extract exhibited inhibitory effects on B. subtilis growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 20.8 mm, 16.7 mm, 14.8 mm, and 12.2 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively.S. aureus is sensitive to the extract at the concentration of 50 mg/ml and very sensitive to the extract at the concentration of 200 mg/ml and 100 mg/ml and extremely sensitive at the concentration of 400 mg/ml
- P. aeruginosa: The hydroethanolic extract of brocchia cinerea showed inhibitory effects against P. aeruginosa, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 13.1 mm, 11.7 mm, 0 mm, and 0 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. P.aeruginosa is sensitive to the extract at the concentration of 400 mg/ml and 200 mg/ml
- *S. aureus*: The extract demonstrated inhibitory effects on S. aureus growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 32.4 mm, 26.2 mm, 24 mm, and 21.7 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. S. aureus is extremely sensitive to the hydroethanolic extract of Brocchia cinerea.
- *Aspergillusbrasiliensis*: The hydroethanolic extract exhibited inhibitory effects against Aspergillus growth. The inhibition zone diameters were 14.9 mm, 14.4 mm, 0 mm, and 0 mm for concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively. *Aspergillus brasiliensis* is very to the extract at the concentration of 400 mg/ml and 200 mg/ml

3.6.1.3. Artemisia Herba alba:

- **.Methanolic extract:**

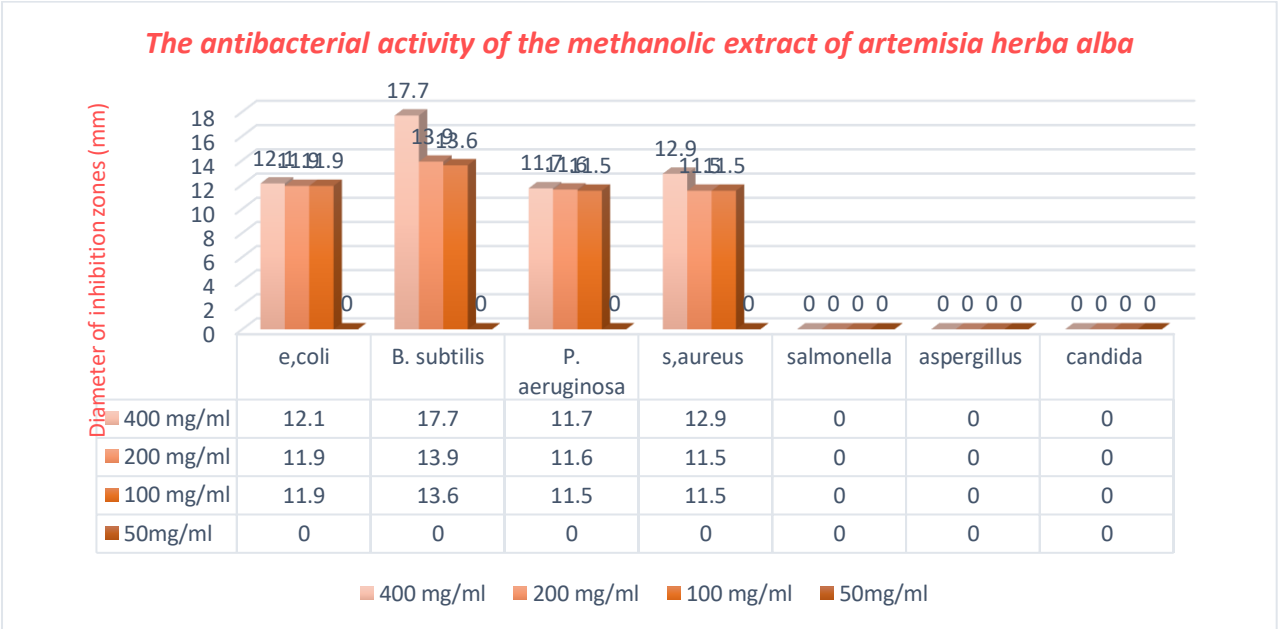


Figure3.33: Antibacterial activity of the methanolic extract of *artemisia herba alba* expressed by zone diameters in mm

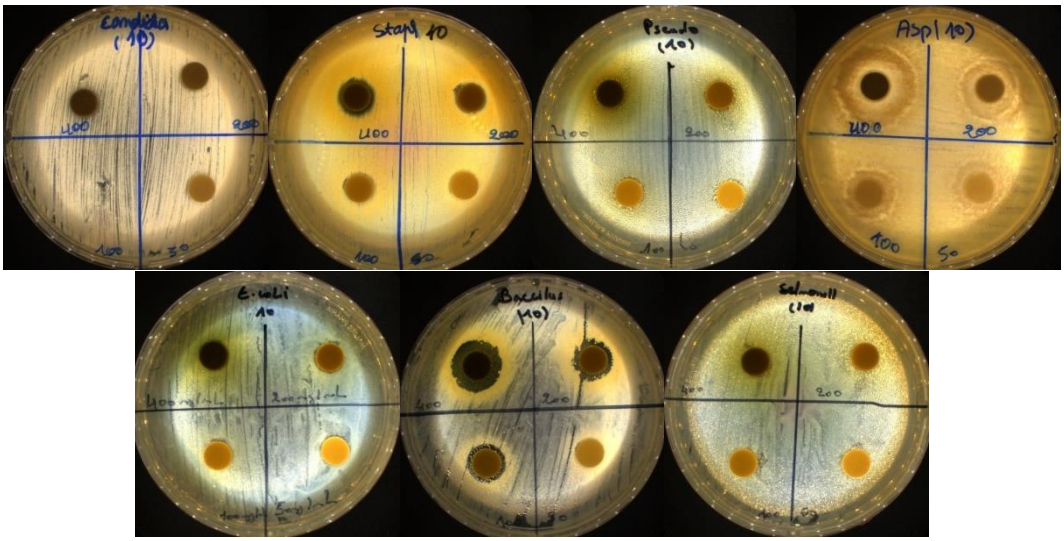


Figure3.34: Results of the antibacterial effect of the methanolic extract of *artemisia herba alba* **(personal photos)**

✓ **Results:**

The methanolic extract of herba alba demonstrated varying levels of inhibitory effects against different strains. They were effective against E. coli, B. subtilis, P. aeruginosa (except at the lowest concentration), and S. aureus. For E. coli and B. subtilis, However, no inhibitory effects were observed against Salmonella, Aspergillus, or Candida.

- *E. coli:* The extract displayed inhibitory effects on B. subtilis growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 17.7 mm, 13.9 mm, and 13.6 mm at concentrations of 400 mg/ml, 200 mg/ml, and 100 mg/ml, respectively. No inhibition was observed at a concentration of 50 mg/ml. E. coli is sensitive to the methanolic extract of artemisia herba alba.
- *B.* subtilis: The extract displayed inhibitory effects on B. subtilis growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 17.7 mm, 13.9 mm, and 13.6 mm at concentrations of 400 mg/ml, 200 mg/ml, and 100 mg/ml, respectively. No inhibition was observed at a concentration of 50 mg/ml. b. subtilis is very sensitive to the methanolic extract of artemisia herba alba at the concentration of 400 mg/ml,200 mg/ml,100 mg/ml and resistant at the concentration of 50mg/ml
- *P.* aeruginosa: The methanolic extract showed inhibitory effects against P. aeruginosa, with similar inhibition zone diameters observed at all concentrations (11.7 mm, 11.6 mm, and 11.5 mm) except for 50 mg/ml, where no inhibition was observed. *P.* aeruginosa is sensitive to the extract for all concentration except 50 mg/ml (resistant).
- *S. aureus*:The methanolic extact of exhibited inhibitory effects on S. aureus growth, with similar inhibition zone diameters observed at all concentrations (12.9 mm, 11.5 mm, and 11.5 mm) except for 50 mg/ml, where no inhibition was observed. S. aureus is resistant to the extract at the concentration of 50 mg/ml and sensitive at the concentration (400 mg/ml, 200 mg/ml .100 mg/ml)

- **Aqueous extract:**

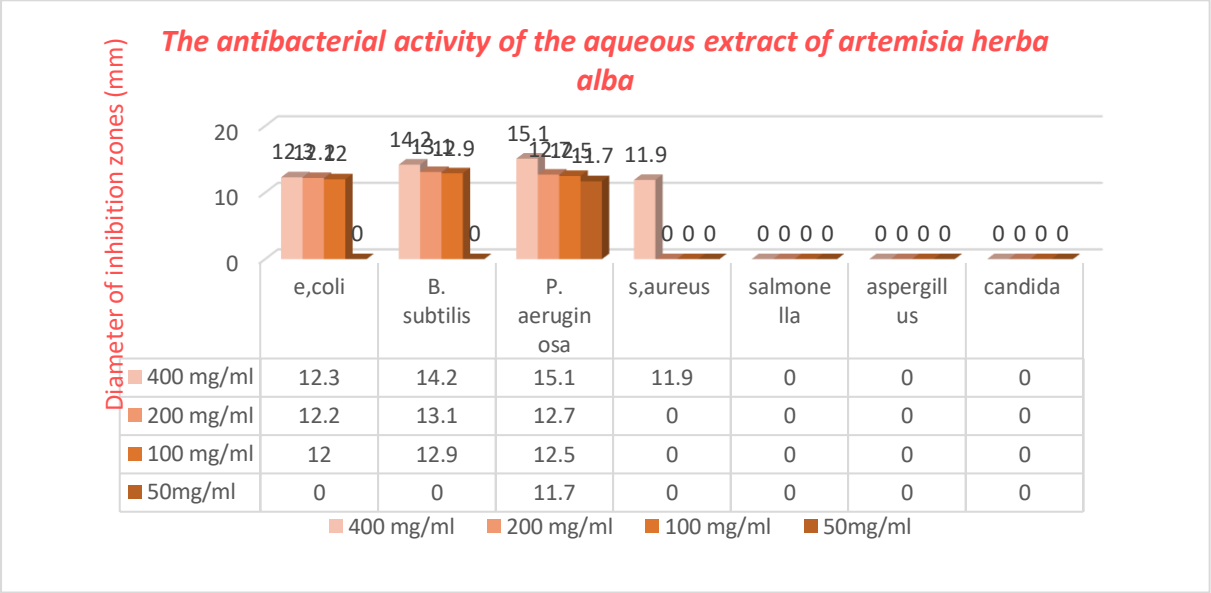


Figure3.35: Antibacterial activity of the aqueous extract of *artemisia Herba alba* expressed by zone diameters in mm

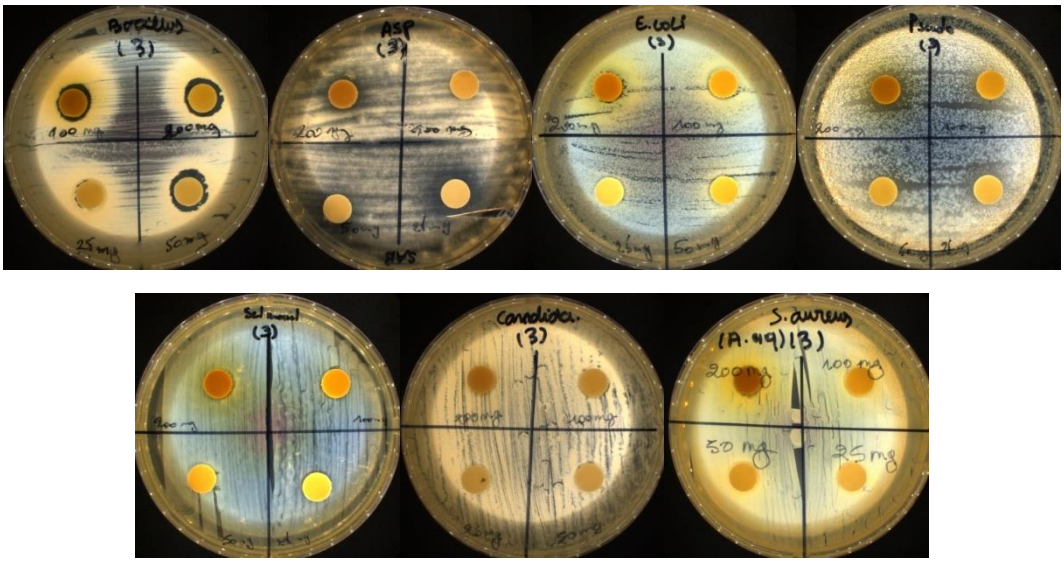


Figure3.36: Results of the antibacterial effect of the aqueous extract of *artemisia Herba alba* **(personal photos).**

✓ **Results:**

The aqueous extract of artemisia herba alba demonstrated varying levels of inhibitory activity against the tested strains. They exhibited the strongest inhibitory effects against P. aeruginosa, followed by E. coli and B. subtilis. S. aureus showed sensitivity only at the highest concentration. No inhibitory effects were observed against Salmonella, Aspergillus, or Candida

- *Salmonella, Aspergillus, Candida* albicans: No inhibitory effects were observed at any of the tested concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml).thisstarin are resistant to the aqueous extract of artemisia herba alba.

- E. coli: The extract displayed inhibitory effects on E. coli growth, with increasing inhibition zone diameters as the concentration increased. The inhibition zone diameters were 12.3 mm, 12.2 mm, and 12.0 mm at concentrations of 400 mg/ml, 200 mg/ml, and 100 mg/ml, respectively. No inhibition was observed at a concentration of 50 mg/ml (resistant).E.coli is sensitive to the aqueous extract of artemisia at the concentration (400 mg/ml,200 mg/ml, 100 mg/ml)
- B. subtilis: the aqueous extract of artemisia showed inhibitory effects against B. subtilis, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 14.2 mm, 13.1 mm, and 12.9 mm at concentrations of 400 mg/ml, 200 mg/ml, and 100 mg/ml, respectively. No inhibition was observed at a concentration of 50 mg/ml (resistant). B. subtilis is sensitive for the other concentration
- P. aeruginosa: The extract exhibited inhibitory effects on P. aeruginosa growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters were 15.1 mm, 12.7 mm, 12.5 mm, and 11.7 mm at concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, respectively.P. aeruginosa is sensitive to the aqueous extract of artemisia at all the concentration except at 400 mg/ml is very sensitive.
- S. aureus: to the aqueous extract of artemisia showed inhibitory effects against S. aureus only at the highest concentration (400 mg/ml), with an inhibition zone diameter of 11.9 mm. No inhibition was observed at lower concentrations (200 mg/ml, 100 mg/ml, and 50 mg/ml). S. aureus is sensitive to the aqueous extract of artemisia herba alba at the concentration of 400 mg/ml and resistant for the other concentration.

- **.<u>Hydroethanolic extract:</u>**

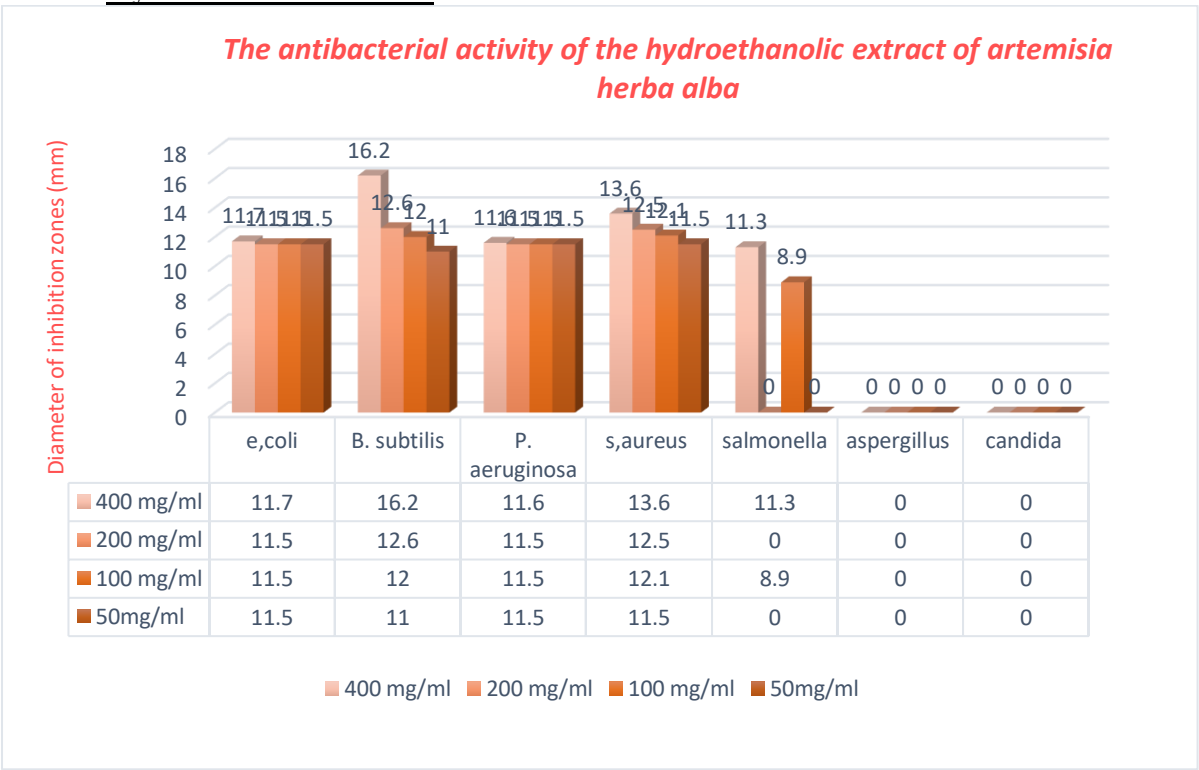


Figure3.37: Antibacterial activity of the hydroethanolic extract of *artemisia herba alba* expressed by zone diameters in mm

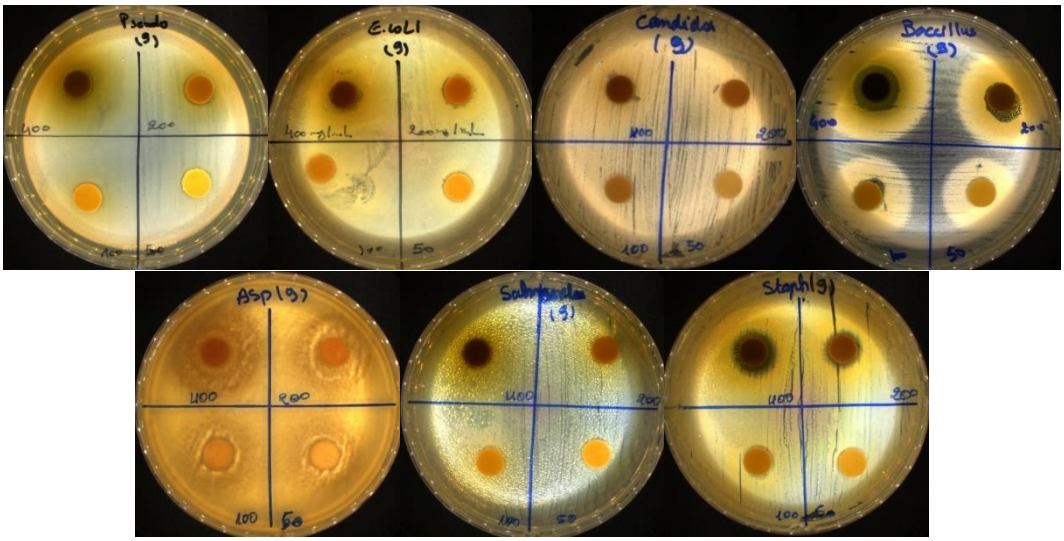


Figure3.38: Results of the antibacterial effect of the hydroethanolic extract of *artemisia herba alba* **(personal photos).**

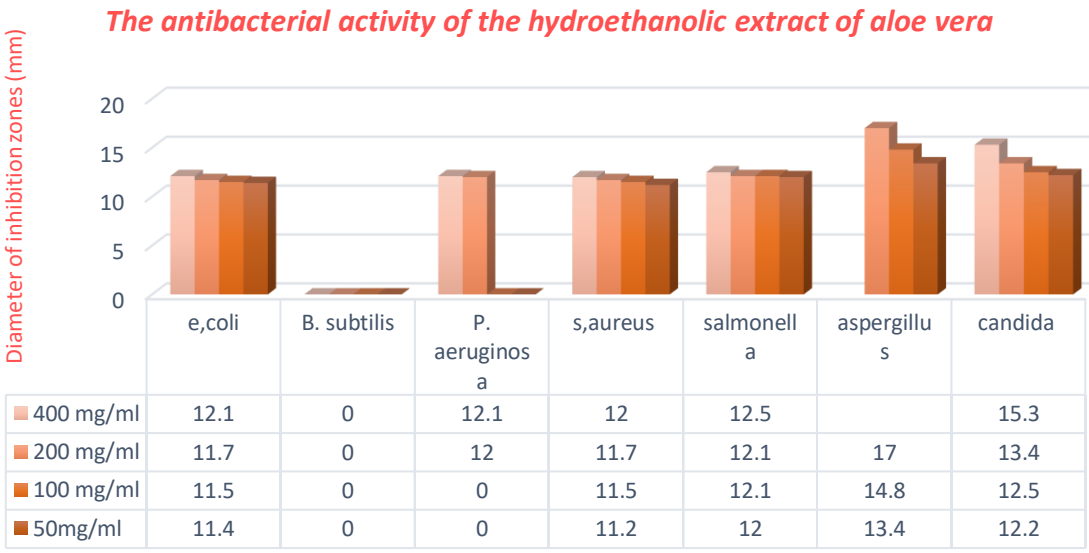
✓ **Resultats:**

The hydroethanolic extract of artemisia herba albademonstrated inhibitory effects against E. coli, B. subtilis, P. aeruginosa, S. aureus, and Salmonella, with varying degrees of effectiveness. The inhibition zone diameters differed among the strains and concentrations tested. No inhibitory effects were observed against Aspergillus or Candida.

- Aspergillus or Candida: A diameter of 0 mm was observed for all concentrations, indicating that the hydroethanolic extract of artemisia herba alba did not inhibit the growth of this strains at any of the tested concentrations. making it resistance to this extract
- E. coli: the hydroethanolic extract of artemisia herba albashowed consistent inhibitory effects on E. coli growth across all tested concentrations. The inhibition zone diameters were around 11.5-11.7 mm, indicating a moderate level of sensitivity.E.coli is sensitive to the extract
- B. subtilis: The extract exhibited inhibitory effects against B. subtilis, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 400 mg/ml with an inhibition zone diameter of 16.2 mm, followed by 12.6 mm at 200 mg/ml, 12.0 mm at 100 mg/ml, and 11.0 mm at 50 mg/ml. b. subtilis is very sensitive to the hydroethanolic extract of artemisia herba alba at the concentration of 400 mg/ml and sensitive to the other concentration.
- P. aeruginosa: The extract showed similar inhibitory effects against P. aeruginosa at all tested concentrations, with inhibition zone diameters ranging from 11.5 mm to 11.6 mm. This indicates a consistent level of sensitivity.
- S. aureus: the hydroethanolic extract of artemisia herba albaexhibited inhibitory effects on S. aureus growth, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 400 mg/ml with an inhibition zone diameter of 13.6 mm, followed by 12.5 mm at 200 mg/ml, 12.1 mm at 100 mg/ml, and 11.5 mm at 50 mg/ml.S.aureus is sensitive to the extract.
- Salmonella: the hydroethanolic extract of artemisia herba albashowed inhibitory effects against Salmonella, with varying inhibition zone diameters at different concentrations. The inhibition zone diameters were 11.3 mm at 400 mg/ml, no inhibition at 200 mg/ml, 8.9 mm at 100 mg/ml, and no inhibition at 50 mg/ml. salmonella is sensitive to the extract at the concentration of 400 mg/ml and resistant for the other concentration

3.6.1.4. Aloe vera:

- **.Hydroethanolic extract:**



	e,coli	B. subtilis	P. aeruginosa	s,aureus	salmonella	aspergillus	candida
400 mg/ml	12.1	0	12.1	12	12.5		15.3
200 mg/ml	11.7	0	12	11.7	12.1	17	13.4
100 mg/ml	11.5	0	0	11.5	12.1	14.8	12.5
50mg/ml	11.4	0	0	11.2	12	13.4	12.2

Figure3.39: Antibacterial activity of the hydroethanolic extract of *aloe vera* expressed by zone diameters in mm

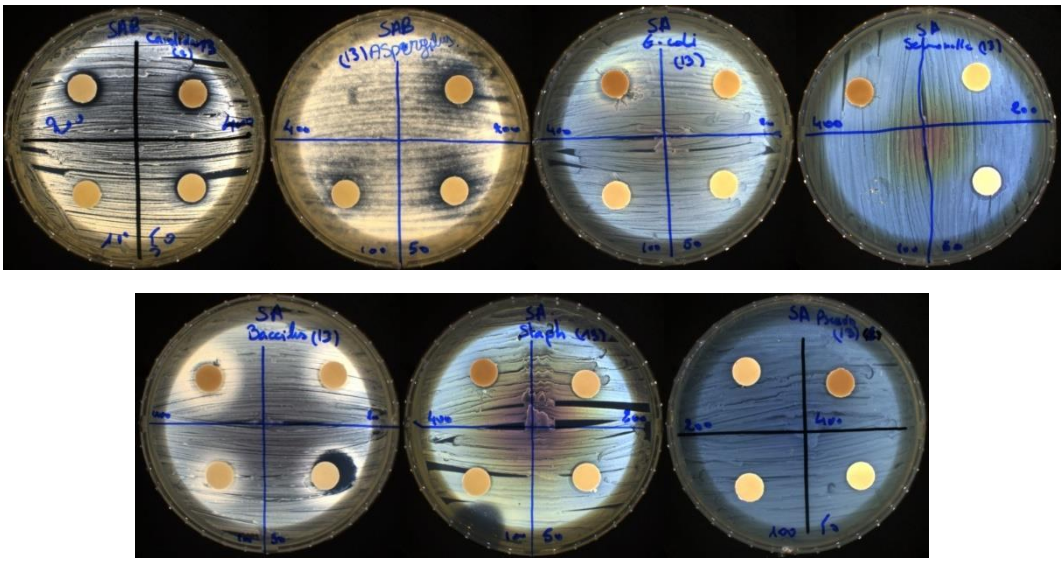


Figure3.40: Results of the antibacterial effect of the hydroethanolic extract of *Aloe vera* **(personal photos).**

✓ **Results:**

The hydroethanolic extract of Aloe vera were tested against different strains, and the following observations were made:

- E. coli: The hydroethanolic extract of Aloe vera exhibited inhibitory effects on E. coli growth, with decreasing inhibition zone diameters as the concentration decreased. The inhibition zone diameters ranged from 11.4 mm at 50 mg/ml to 12.1 mm at 400 mg/ml.

- B. subtilis: No inhibitory effects were observed against B. subtilis at any of the tested concentrations. The inhibition zone diameters were all 0 mm, indicating no sensitivity (resistant) to the substances.
- P. aeruginosa: the extract displayed inhibitory effects on P. aeruginosa growth, with the highest inhibition observed at 12.1 mm at 400 mg/ml and 12.0 mm at 200 mg/ml. No inhibition was observed at 100 mg/ml and 50 mg/ml.P. aeruginosa is sensitive to Thehydroethanolic extract of Aloe vera at the concentration (400 mg/ml ,200 mg/ml)and resistant at the concentration of 100 mg/ml and 50 mg/ml.
- S. aureus: The extract exhibited inhibitory effects on S. aureus growth, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 12.0 mm at 400 mg/ml, followed by 11.7 mm at 200 mg/ml, 11.5 mm at 100 mg/ml, and 11.2 mm at 50 mg/ml. S. aureus is sensitive to the hydroethanolic extract of Aloe vera
- Salmonella: The extract showed inhibitory effects against Salmonella, with similar inhibition zone diameters observed across the tested concentrations. The inhibition zone diameters ranged from 12.0 mm to 12.5 mm.
- Aspergillus: the hydroethanolic extract of Aloe vera displayed inhibitory effects on Aspergillus growth, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 17.0 mm at 400 mg/ml, followed by 14.8 mm at 200 mg/ml, and 13.4 mm at 100 mg/ml. aspergillus is very sensitive to the extract at the concentration of 400 mg/ml and 200 mg/ml
- Candida: The hydroethanolic extract of Aloe vera exhibited inhibitory effects on Candida growth, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 15.3 mm at 400 mg/ml, followed by 13.4 mm at 200 mg/ml, 12.5 mm at 100 mg/ml, and 12.2 mm at 50 mg/ml. candida albicans is very sensitive to the extract at the concentration of 400 mg/ml and sensitive at the other concentration

- **Aloe vera pure gel:**

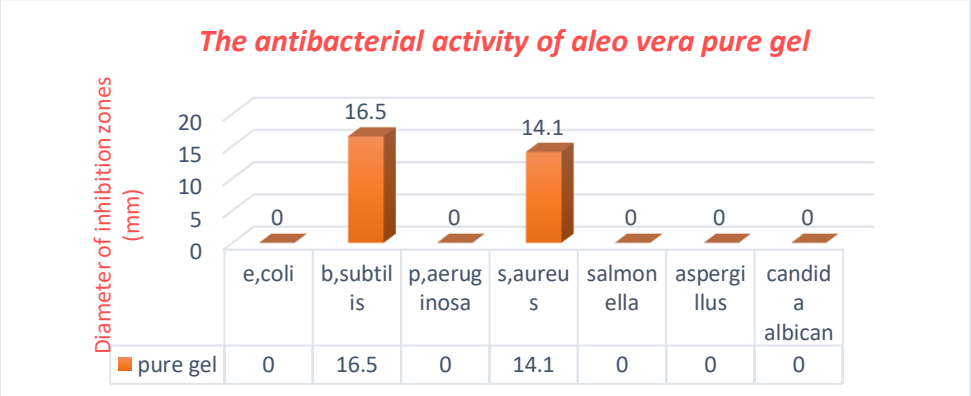


Figure3.41: Antibacterial activity of *aloe vera* pure gel expressed by zone diameters in mm

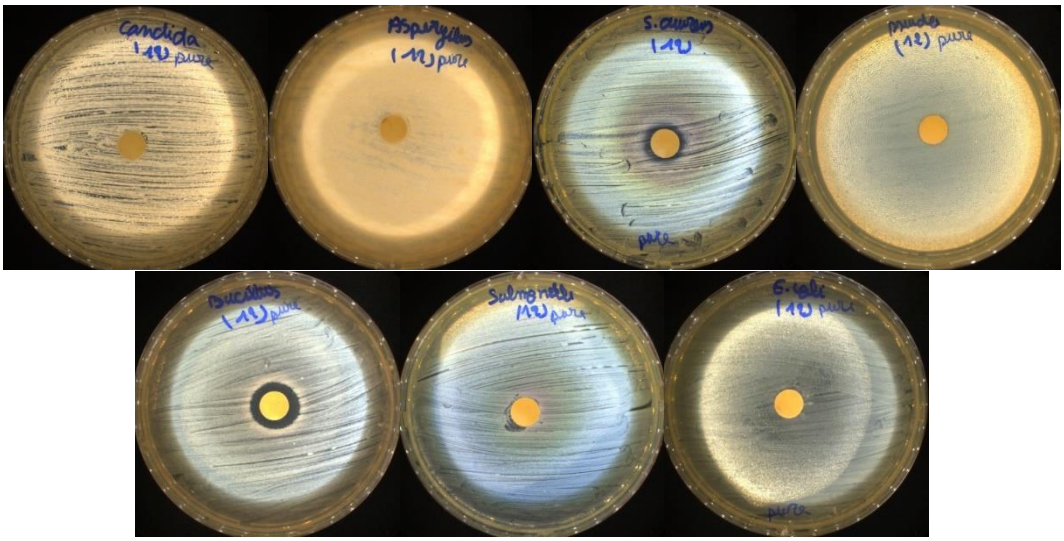


Figure3.42: Results of the antibacterial effect of pure gel of *Aloe vera* **(personal photos).**

✓ **Results:**

It appears that the pure gel substance was tested against various strains.

- E. coli, P. aeruginosa, Salmonella, Aspergillus, and Candida albicans: no inhibitory effects were observed which mean this strain has a resistant to the pure gel of aloe vera
- B. subtilis: The pure gel exhibited inhibitory effects on B. subtilis, with an inhibition zone diameter of 16.5 mm. This indicates sensitivity of B. subtilis to the pure gel substance.B. subtilis is very sensitive to the pure gel of aloe vera
- S. aureus: The pure gel exhibited inhibitory effects on S. aureus, with an inhibition zone diameter of 14.1 mm. This indicates sensitivity of S. aureus to the pure gel substance. S. aureus is very sensitive to the pure gel of aloe vera.

- **.Pure Aloe Vera gel dilutions :**

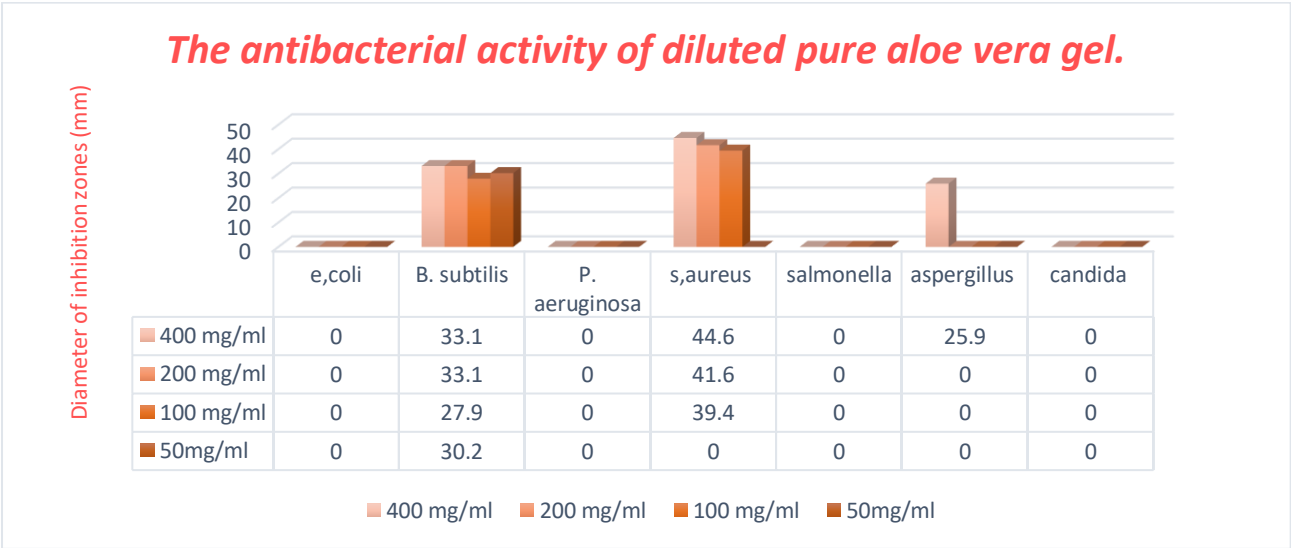


Figure3.43: Antibacterial activity of diluted pure aloe vera gel expressed by zone diameters in mm

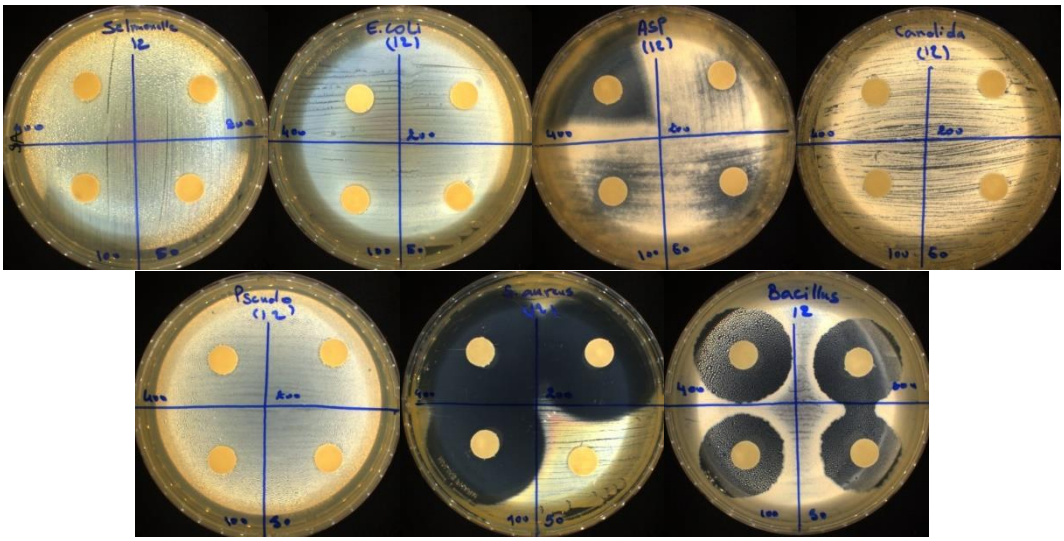


Figure3.44: Results of the antibacterial effect of diluted pure aloe vera gel**(personal photos).**

✓ ***Results:***

The results of the antimicrobial effect of diluted pure aloe vera gel are interpreted as follows:

- B. subtilis: The substance showed inhibitory effects on B. subtilis growth, with varying degrees of sensitivity depending on the concentration. The highest inhibition was observed at 33.1 mm at 400 mg/ml and 200 mg/ml, followed by 27.9 mm at 100 mg/ml, and 30.2 mm at 50 mg/ml.
- S. aureus: The substance showed inhibitory effects on S. aureus growth, with decreasing inhibition zone diameters as the concentration decreased. The highest inhibition was observed at 44.6 mm at 400 mg/ml, followed by 41.6 mm at 200 mg/ml, and 39.4 mm at 100 mg/ml. No inhibition was observed at 50 mg/ml.
- Aspergillus: The substance showed inhibitory effects on Aspergillus growth, with the highest inhibition observed at 25.9 mm at 400 mg/ml. No inhibition was observed at the other tested concentrations.
- No inhibitory effects were observed on E. coli, P. aeruginosa, Salmonella, and Candida.

3.6.1.5.Brocchia cinerea essential oil:

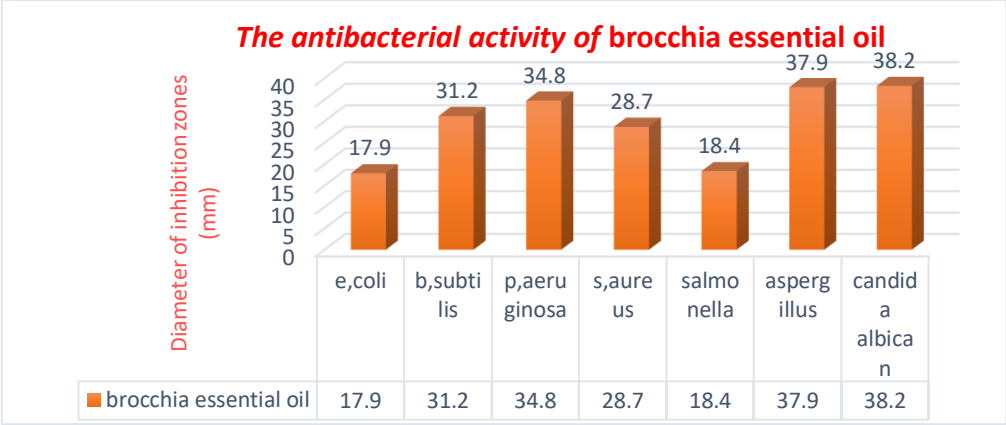


Figure3.45: Antibacterial activity of *Brocchia cinerea* essential oil expressed by zone diameters in mm

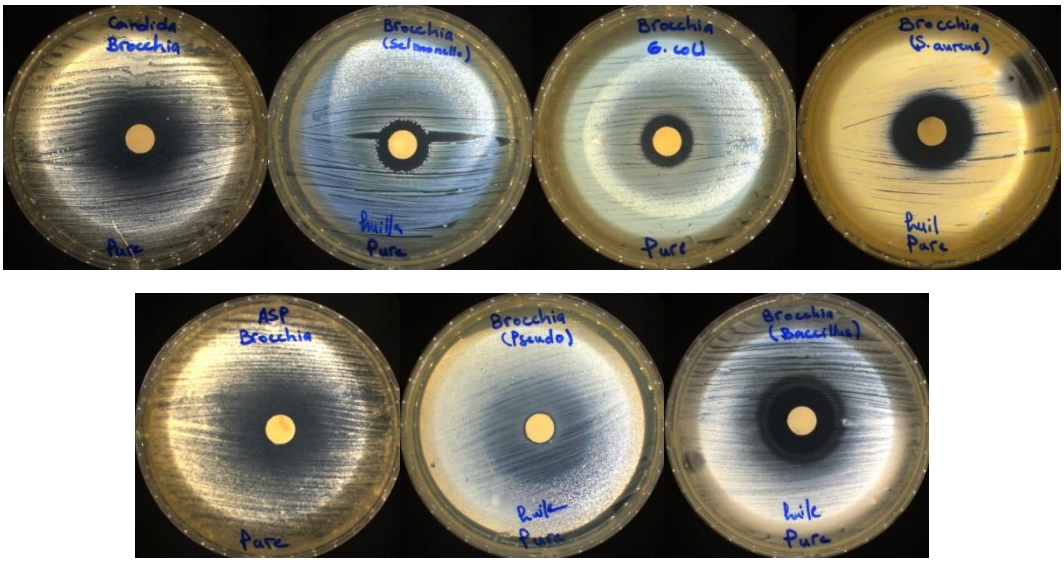


Figure3.46: Results of the antibacterial effect of *Brocchia cinerea* essential oil(**personal photos)**

✓ **Results :**

The inhibitory effects of Brocchia essential oil on various microbial strains were evaluated by measuring the inhibition zone diameters. The results indicate the following:

- E. coli: Brocchia essential oil exhibited a significant inhibitory effect on E. coli, with an inhibition zone diameter of 17.9 mm. This suggests that the essential oil has potential antimicrobial properties against E. coli.
- B. subtilis: The essential oil showed a strong inhibitory effect on B. subtilis, with an inhibition zone diameter of 31.2 mm. This indicates that Brocchia essential oil is effective in inhibiting the growth of B. subtilis.
- P. aeruginosa: Brocchia essential oil demonstrated a considerable inhibitory effect on P. aeruginosa, with an inhibition zone diameter of 34.8 mm. This suggests that the essential oil has potent antimicrobial properties against P. aeruginosa.
- S. aureus: The essential oil exhibited a moderate inhibitory effect on S. aureus, with an inhibition zone diameter of 28.7 mm. This indicates that Brocchia essential oil has the potential to inhibit the growth of S. aureus.
- Salmonella: Brocchia essential oil displayed an inhibitory effect on Salmonella, with an inhibition zone diameter of 18.4 mm. This suggests that the essential oil may have antimicrobial activity against Salmonella.
- Aspergillus: The essential oil demonstrated a strong inhibitory effect on Aspergillus, with an inhibition zone diameter of 37.9 mm. This indicates that Brocchia essential oil has potent antifungal properties against Aspergillus.
  Candida albicans: Brocchia essential oil showed significant inhibitory effects on Candida albicans, with an inhibition zone diameter of 38.2 mm. This suggests that the essential oil has strong antifungal properties against Candida albicans.

3.6.1.6.Artemisia Herba_Alba essential oil:

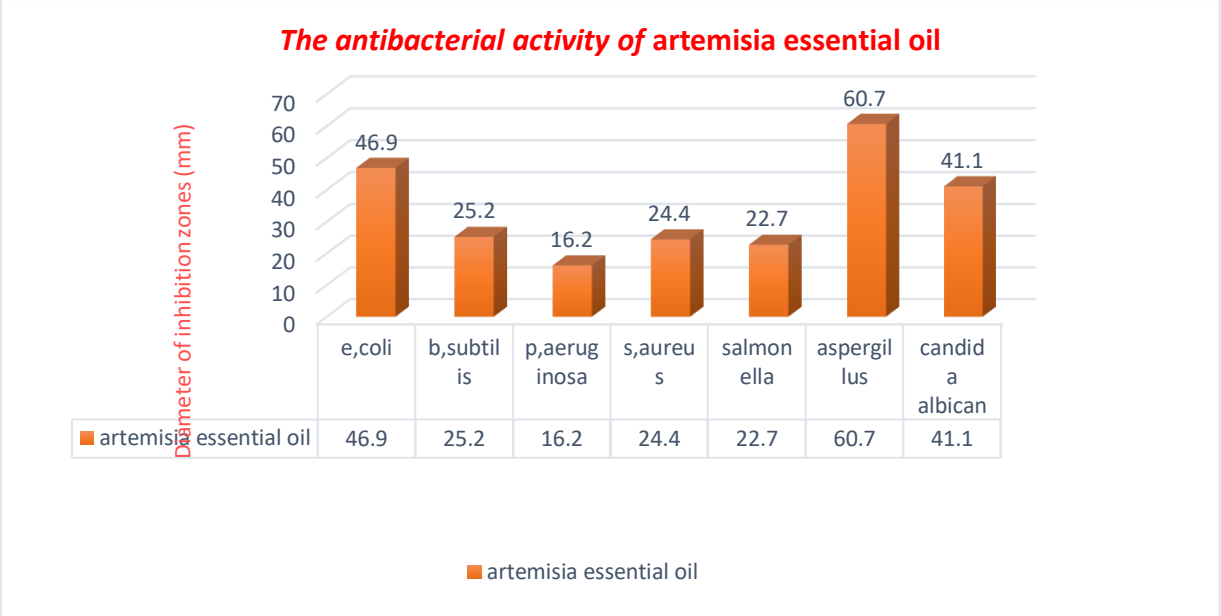


Figure3.47: Antibacterial activity of *artemisia Herba_Alba* essential oil expressed by zone diameters in mm

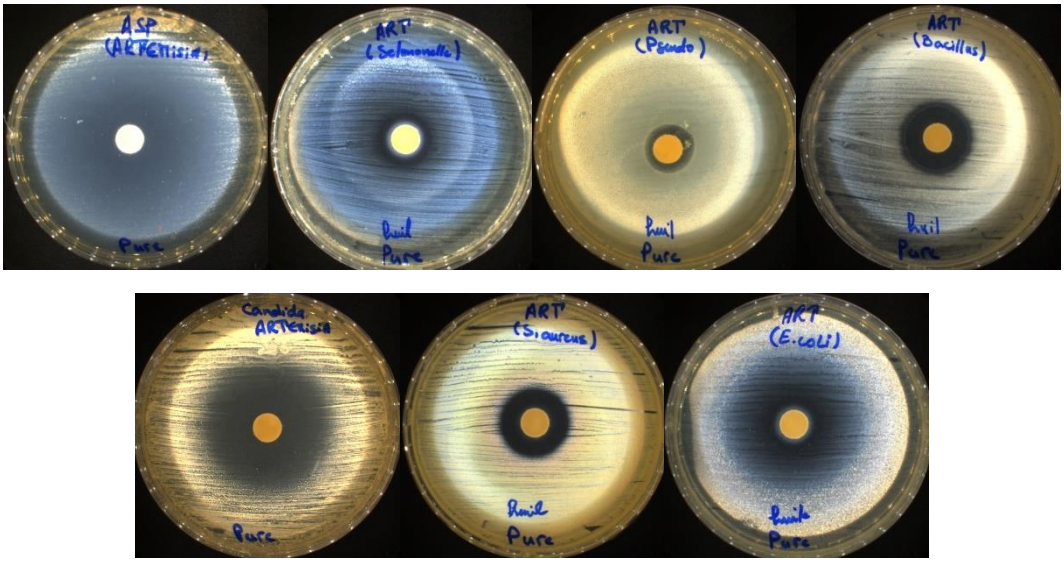


Figure3.48: Results of the antibacterial effect of *Artemisia Herba_Alba* essential oil**(personal photos)**

- ✓ **Results:**

The inhibitory effects of Artemisia essential oil on various microbial strains were evaluated by measuring the inhibition zone diameters. The results indicate the following:

- E. coli: Artemisia essential oil exhibited a strong inhibitory effect on E. coli, with an inhibition zone diameter of 46.9 mm. This suggests that the essential oil has potent antimicrobial properties against E. coli.

- B. subtilis: The essential oil showed a moderate inhibitory effect on B. subtilis, with an inhibition zone diameter of 25.2 mm. This indicates that Artemisia essential oil has the potential to inhibit the growth of B. subtilis.
- P. aeruginosa: Artemisia essential oil demonstrated a relatively low inhibitory effect on P. aeruginosa, with an inhibition zone diameter of 16.2 mm. This suggests that the essential oil may have limited antimicrobial activity against P. aeruginosa.
- S. aureus: The essential oil exhibited a moderate inhibitory effect on S. aureus, with an inhibition zone diameter of 24.4 mm. This indicates that Artemisia essential oil has the potential to inhibit the growth of S. aureus.
- Salmonella: Artemisia essential oil displayed a moderate inhibitory effect on Salmonella, with an inhibition zone diameter of 22.7 mm. This suggests that the essential oil may have antimicrobial activity against Salmonella.
- Aspergillus: The essential oil demonstrated a strong inhibitory effect on Aspergillus, with an inhibition zone diameter of 60.7 mm. This indicates that Artemisia essential oil has potent antifungal properties against Aspergillus.
- Candida albicans: Artemisia essential oil showed significant inhibitory effects on Candida albicans, with an inhibition zone diameter of 41.1 mm. This suggests that the essential oil has strong antifungal properties against Candida albicans

3.7. Results of the creams for burns and wounds :

3.7.1.The first cream : aloe vera with pure gel

3.7.1.1. Observation of Burns (first rabbit):

During the 15-day observation period in rabbits, the burns were meticulously assessed and described every 4 days by seeing the changes of the color and texture and wound surface in the left side of the burn where the treatment and the application of the aloe vera cream was and the right side without the treatment or any application of the cream , The following is a summary of the observations made during each assessment.

- **Day 1 (Initial Burn): The first rabbit :**

- A circle-shaped burn(third degree) was observed on the rabbit's leg , characterized by extensive tissue damage.
-The skin in the burned area exhibited signs of heat damage, had a deep, blackened appearance.
-The burn site showed signs of a dry, leathery texture.
-Hair follicles within the burned area were completely destroyed.
-Mild swelling were present around the burn.
-The texture of the burned skin was irregular and rough to the touch.



Figure 3.49 : The first day of the burn (left side of the first rabbit)(**personal photo 2023)**



Figure 3.50: The first day of the burn (right side of the first rabbit)(**personal photo 2023)**

- **<u>Day 4 of the treatment (left side):</u>**

The rabbit received appropriate treatment for the third-degree burn with the aloe vera cream (pure gel):
-The third-degree burn remained unchanged in appearance from Day 1
-The blackened, charred skin persisted with a dry and leathery texture
.-The skin in the burned area appeared more fragile and delicate
-No signs of healing or improvement were observed.
-Swelling and inflammation around the burn site have increased.



Figure 3.51: Day 4 of the burn **(personal photo)**

- **Day 4 without the treatment (the right side):**

The burned area remained blackened with a dry, leathery texture.

-No visible signs of healing or tissue regeneration were observed.

- **Day 8 of the treatment (left side):**

After eight days of treatment with aloe vera cream in the left side there were noticeable changes in the rabbit's burn wound :
-The darker color of the burn had started to fade, becoming lighter and less pronounced. transitioning from black to a red.
-Some areas showed signs of blistering and the formation of fluid-filled sacs.
-The texture of the burned skin remained rough, with the presence of a red scab formation.
-The skin in the burned area appeared more fragile and delicate.
-The texture of the burned skin was rough and scaly.
-swelling were present around the burn.
-Hair growth around the burned area was minimal.

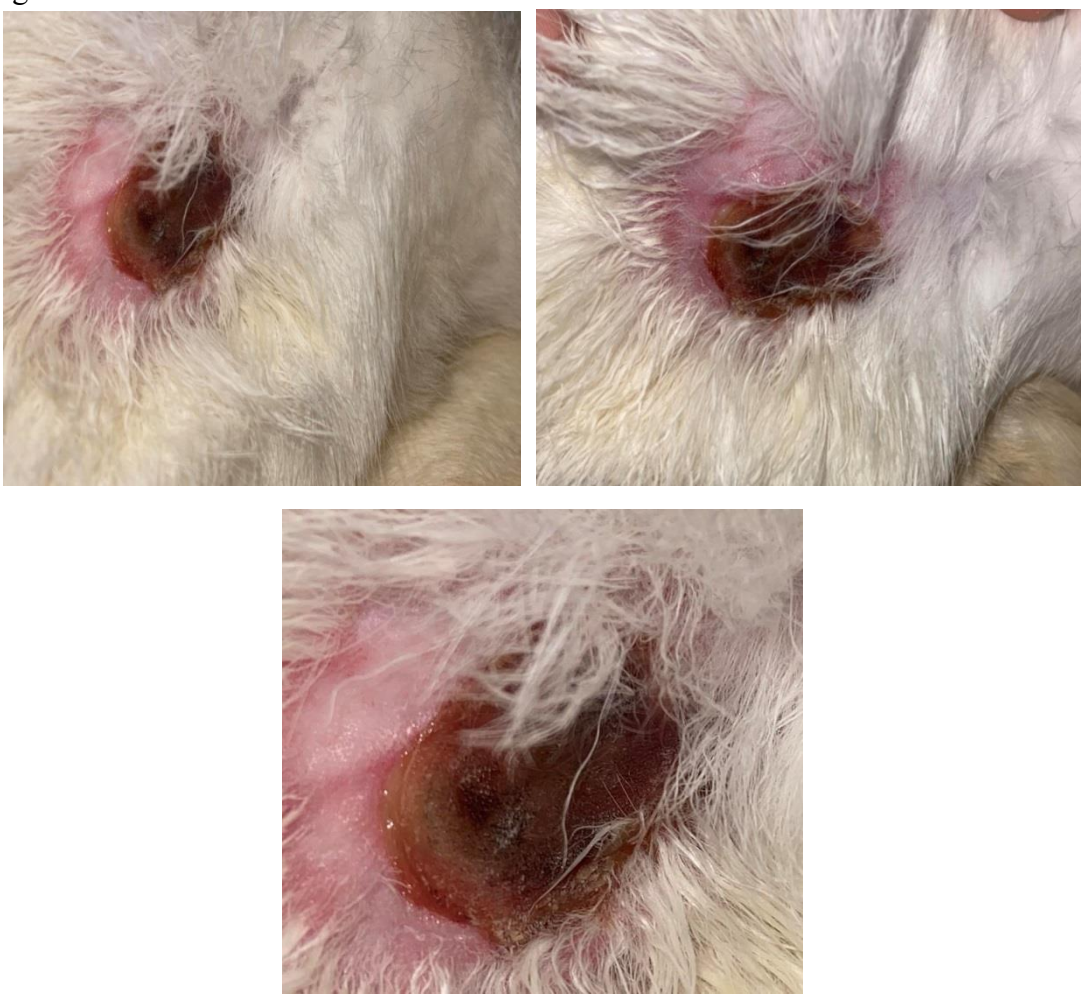


Figure 4.52 : Day 8 of treatment with aloe vera cream **(personal photo 2023)**

- ✓ **Day 8 without the treatment of the right side:**

-For the right side of the rabbit the burn remained unchanged in appearance from Day 1.

-The burned area remained blackened with a dry, leathery texture.

-No visible signs of healing or tissue regeneration were observed.



Figure 3.53: Day 8 of the burn in the right side of the rabbit (without treatment) **(personal photo)**

- **Day 12 of the treatment (left side):**

After 12 days of treatment The the size of the burn was significantly diminished . further improvement was observed in the burn wounds of the rabbits ,Healing wounds were closing, with the formation of scabs. The localized inflammation had subsided (with the disappearance of fluid-filled sacs),, and the granulation tissue appeared more developed and healthy. Sloughing of necrotic tissue continued, and some areas showed signs of reepithelialization. However, the central portion of the burn still displayed considerable depth and exhibited signs of ongoing tissue damage.

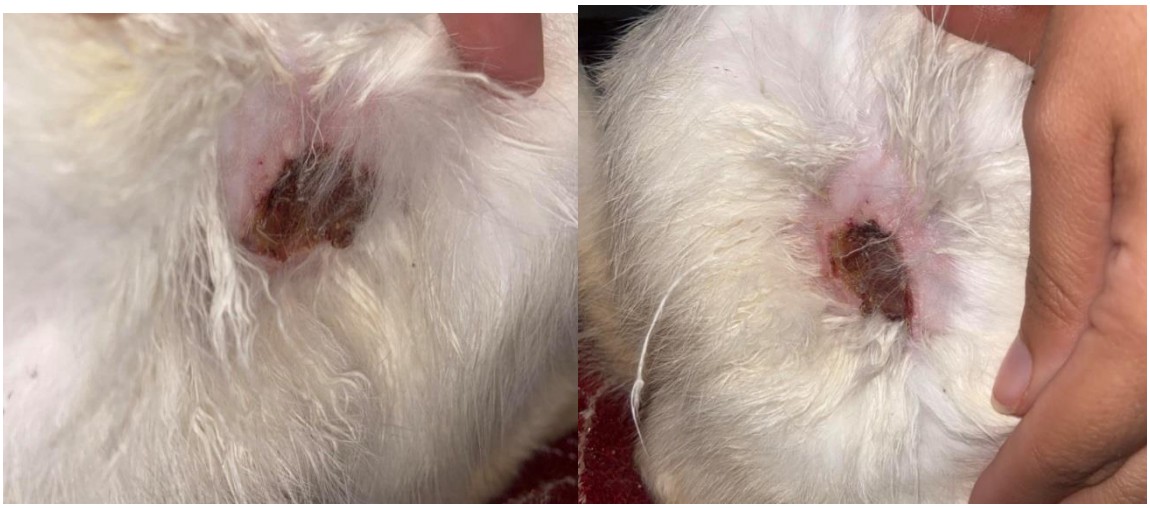




Figure 3.54 : Day 12 of the treatment in the left side of the rabbit **(personal photo 2023)**

- **Day 12 (the right side) without the treatment :**

-The third-degree burn remained largely unchanged throughout the 12-day observation period.

-The burned area continued to exhibit a blackened appearance with a dry, leathery texture.

-No significant signs of healing or tissue regeneration were observed.

-Swelling and inflammation may have persisted



Figure 3.55 : Day 12 of the burn (without the treatment) right side **(personal photo 2023)**

- **Day 15 of the treatment :**

By the end of the 15-day treatment period, the rabbits' burn wounds demonstrated substantial improvement Whereas the size of the burn diminished from the periphery. Inflammation had greatly subsided, and the granulation tissue had filled a significant portion of the wound. The reepithelialization process had progressed further, covering a larger surface area..



Figure 3.56: Day 15 of the treatment with the aloe vera cream in the left side of the rabbit **(personal photo 2023)**

- **Day 15 without the treatment (the right side) :**

-The severe burn stayed the same over the course of 15 days.
-It remained blackened and dry, with a tough, leathery feel.
-There were no significant signs of healing or new tissue growth.

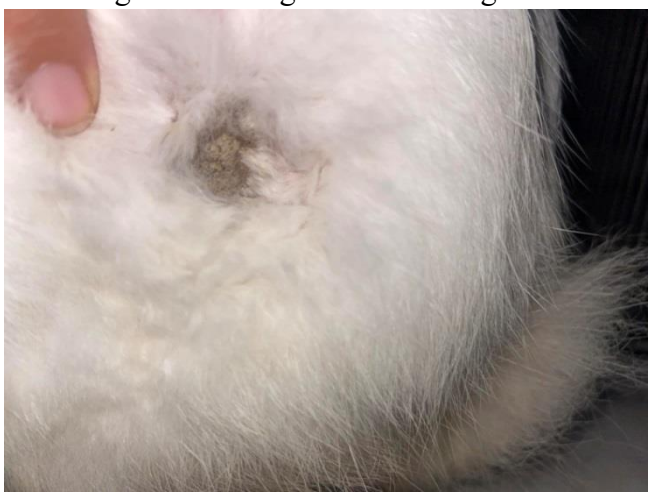


Figure 3.57 : Day 15 of the burn without the treatment (right side**(personal photo 2023))**

3.7.1.2.Evaluation of wounds (the second rabbit):

During the 15-day observation period in rabbits, the wounds were meticulously assessed and described every 4 days by seeing the changes of the color and texture and wound surface in the left side of the burn where the treatment and the application of the aloe vera cream was and the right side without the treatment or any application of the cream , The following is a summary of the observations made during each assessment:

- **Day 1:**

On the first day of the small wound injury in the rabbit, appeared as a fresh injury ,The wound site appeared as a small, superficial injury on the rabbit's skin It exhibited redness, inflammation, and localized swelling.with visible disruption to the normal skin structure. the

damage range was a small cut The wound have shown signs of bleeding, with the presence of blood .



Figure 3.58: The first day of the wound injury (left side of the second rabbit) **(personal photo 2023)**



Figure 3.59: The first day of the wound injury (right side of the second rabbit) **(personal photo 2023)**

- **Day 4 of the treatment (left side) :**

-After four days of the application of aloe vera cream , there were signs of initial healing in the wound. --The redness and inflammation around the wound had started to subside.
-The surrounding skin might still exhibit mild redness or inflammation.
–The size and depth of the wounds have reduced slightly.
-The wound have shown evidence of early granulation tissue formation, with the appearance of pinkish or reddish tissue filling the wound bed. A small scab have formed over the wound, indicating the beginning of the healing process .



Figure 3.60: Day 4 of the treatment of wound injury (left side) **(personal photo 2023)**

- **Day 4 : the right side without the treatment :**

The wound injury remained largely unchanged
-The wound area continued to exhibit a inflammation and redness.
-No significant signs of healing or tissue regeneration were observed.

- **Day 8 of the treatment (left side) :**

By this point, the wound was further progressing in the healing process.about 90% ,The size of the wounds further reduced compared to Day 4. Scabs on the wounds have started to detach or shrink.The inflammation had significantly reduced, and the granulation tissue had developed further, filling a larger portion of the wound. . The surrounding skin have shown minimal redness or inflammation.



Figure 3.61: Day 8 of the treatment on the left side **(personal photo 2023)**

- **Day 8 on the right side (without the treatment of aloe vera cream):**

There was a diffrence between the wound of the left and the right :

Delayed Healing: The wound displayed a slow healing process due to the absence of treatment, resulting in a prolonged closure of the wound.comparing to the wound of the left side with the application of the treatment . The swelling surrounding the wound was noticeably more pronounced in comparison to the treated wound. The absence of treatment enables the inflammation to advance, consequently causing an escalation in swelling.



Figure 3.62: Day 8 without the treatment **(personal photo 2023)**

- **<u>Day 12 of the treatment (left side) :</u>**

By the end of the 12-day period, the wound had shown substantial and earliar progress in healing. The inflammation around the wound had resolved 100% The wound has disappeared also 100% . The healing tissue have appeared pink and healthy, with signs of reepithelialization with Total scar disappearance 100% .



Figure 3.63 : The final result of the wound healing 100% (day 12) **(personal photo 2023)**

- **<u>Day 12: the right side (without the treatment) :</u>**

A small scab have formed over the wound, indicating the beginning of the healing process. The wound shown a slow healing comparing to the other side and delayed closure of the wound.



Figure 3.64: The beginning of the healing process of the wound without treatment**.(personal photo 2023)**

- **<u>Day 15 of the treatment (the left side):</u>**

The treatment was discontinued on day 12 because the wound had completely healed with a 100% recovery rate and total disappearance of any scar.

- **<u>Day 15 without the treatment (the right side) :</u>**

The wound is in initial stage showed that after 12 days the wound started to heal , we observed a long periode of healing in the right side without the treatment comparing to the other side with the treatment



Figure3.65 : Day 15 of the wound without the treatment **(personal photo 2023)**

3.7.2. The second cream : aloe vera with active Ingredient :

3.7.2.1. Observation of Burns (the third rabbit):

During the 15-day observation period in rabbits, the burns were meticulously assessed and described every 4 days by seeing the changes of the color and texture and wound surface in the left side of the burn where the treatment and the application of the aloe vera cream(active ingredient) was and the right side without the treatment or any application of the cream , The following is a summary of the observations made during each assessment.

- **Day 1: (with and without the treatment in both sides) :**

On day 1 of the burning incident, a black second-degree burn is observed on the rabbit. The following description can be provided:

The affected area of the rabbit's skin appears blackened, indicating a severe burn injury. The burn is classified as a second-degree burn, indicating damage to the outer layer of the skin (epidermis) .

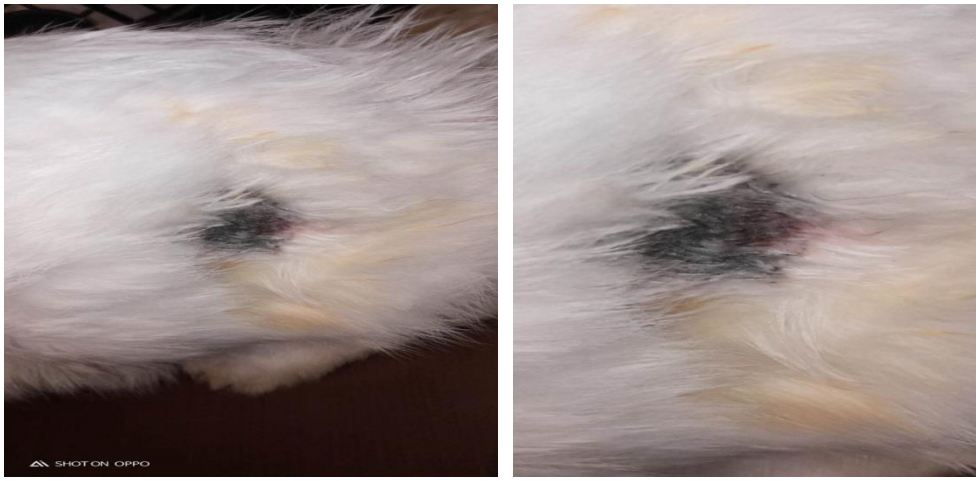


Figure3.66 : The first day of the burn (the left side of the third rabbit) **(personal photo 2023)**



Figure 3.67: The first day of the burn (the right side of the third rabbit) **(personal photo 2023)**

- **Day 4 of the treatment :**

The rabbit received appropriate treatment for the second degree burn with the aoe vera cream (active ingredient):

- The blackened appearance of the burn site persist, but the treatment aims to facilitate healing.

The blackened, charred skin persisted with a dry and leathery texture

.-The skin in the burned area appeared more fragile and delicate

-No signs of healing or improvement were observed.

-Swelling and inflammation around the burn site have increased.

-The burn blisters have increased in size or number.



Figure 3.68: Day 4 of the burn with the treatment in the left side of the third rabbit **(personal photo 2023)**

- **Day 4 without the treatment (the right side) :**

-The burned area remained blackened with a dry, leathery texture.
-No visible signs of healing or tissue regeneration were observed.



Figure 3.69 : Day 4 of the burn (without the treatment of the right side) **(personal photo 2023)**

- **Day 8 of the treatment (the left side) :**

After eight days of treatment with aloe vera cream in the left side there were noticeable changes in the rabbit's burn wound :

-The burn area shows signs of healing with the formation of new tissue.
-the dissepearnce of the black color in thr burn .
-red Scabs have formed over the previously blistered areas .
-The redness and swelling further reduce .
-The exposed tissue appear raw and pink.
-The redness and swelling gradually subside.

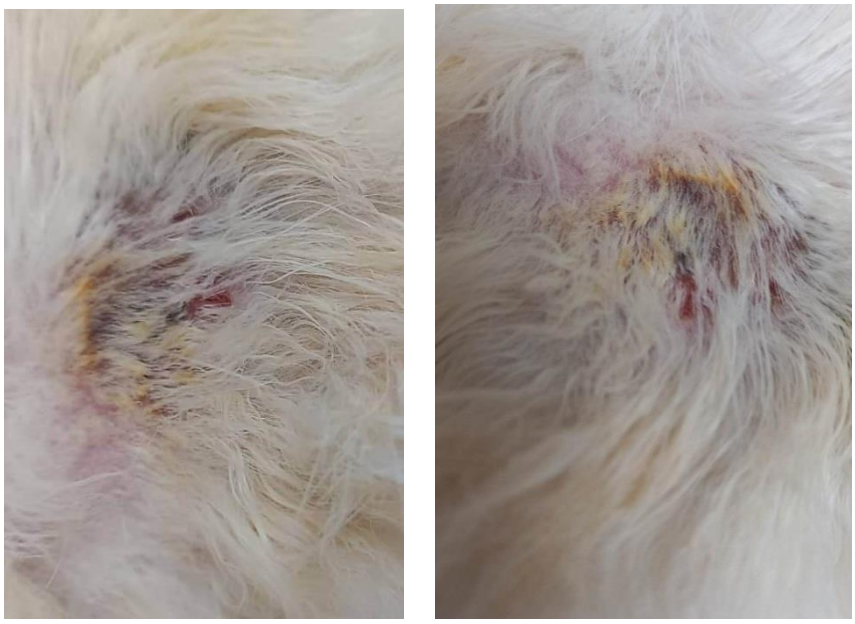


Figure 3.70: Day 8 of the left side burn (with the treatment) **(personal photo 2023)**

- **Day 8 without the treatment (the right side) :**

-On the right side of the rabbit, the burn maintained a consistent appearance throughout the 8-day period.

-The burn site continued to exhibit a blackened appearance, resembling a dry and leathery texture.

-No apparent changes or improvements in the burn's appearance were observed.

-There were no visible signs of healing or the regeneration of new tissue in the affected area.



Figure 3.71: Day 8 of the right side burn (without the treatment) **(personal phot 2023)**

- **Day 12 of the treatment (the left side) :**

-After 12 days of the treatment with the second aloe vera cream :

-The burn area shows signs of gradual healing.
-The blackened tissue begins to lighten, indicating the regeneration of healthy skin.
-Blisters may have started to shrink or dry up.
-Blisters may have completely dried or flattened, forming red scabs.
-The burn site shows signs of new tissue formation and granulation



Figure 3.72: Day 8 of the burn treatment (the left side of the third rabbit) **(personal photo 2023)**

- **Day 12 without the treatment (the right side) :**

On the right side of the rabbit, the burn maintained a consistent appearance throughout the 12-day period.
The burn site continued to exhibit a blackened appearance, resembling a dry and leathery texture.
No apparent changes or improvements in the burn's appearance were observed.
There were no visible signs of healing or the regeneration of new tissue in the affected area.



Figure 3.73: Day 12 of the burn without the treatment (right side) **(personal photo 2023)**

- **Day 15 of the treatment (left side) :**
- **After 15 days of the application of aloe vera cream :**

-Significant healing has occurred in the burn area. Whereas the size of the burn diminished from the periphery.

-The blackened tissue has largely disappeared, replaced by healthy, pinkish skin.

-Scabs may still be present as the remaining signs of the previously formed blisters.

-The rabbit experiences minimal pain and discomfort, thanks to the effective burn cream treatment.



Figure 3.74 : Day 15 of the treatment (left side **(personal photo 2023)**

- **Day 15 without the treatment (the right side):**



Figure 3.75: Day 15 of the wound without the treatment(right side) **(personal photo 2023)**

3.7.2.2. Evaluation of wounds (the forth rabbit):

During the 15-day observation period in rabbits, the wounds were meticulously assessed and described every 4 days by seeing the changes of the color and texture and wound surface in the left side of the burn where the treatment and the application of the aloe vera cream was and the right side without the treatment or any application of the cream , The following is a summary of the observations made during each assessment.

- **Day 1 of the wound injury (both sides):**

A deep wound injury is observed in the rabbit, resulting from the operation performed in the lab.The wound exhibits visible tissue damage, with irregular and jagged edges with the signs of Bleeding with fresh blood .



Figure 3.76 : The first day of the wound injury (the left side) **(personal photo)**

- **Day 4 of the treatment (the left side of the rabbit):**

After four days of the application of aloe vera cream (active ingredient). The wound treated with wound cream may show signs of reduced inflammation, with diminished redness and swelling and Granulation tissue continues to develop, indicating a progressing healing process



Figure 3.77: Day 4 of the wound injury (with the application of the treatment **(personal photo 2023))**

- **Day 4 : without the treatment (the right side) :**

The wound shows signs of increased inflammation, with redness and swelling in the surrounding area. Granulation tissue start forming, but the healing process was slower compared to the treated wound in a long periode .

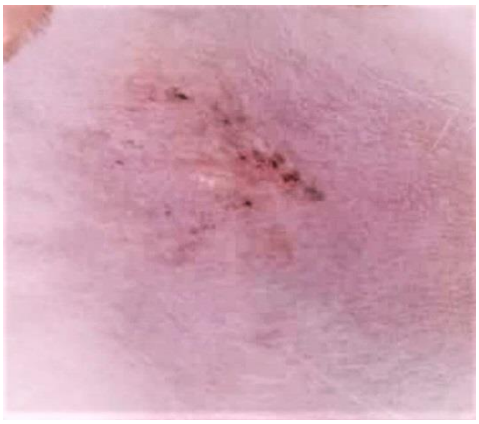


Figure 3.78: day 8 of the wound (right side) without the treatment **(personal photo 2023)**

- **Day 8 of the wound treatment (left side):**

- The treated wound shows substantial progress in closure, with the edges app roximating more closely.
-The granulation tissue becomes more prominent, filling the wound gap effectively.
-The Inflammation continues to diminish, with reduced redness and swelling.
- No bleeding is observed, indicating improved wound stability.



Figure 3.79: Day 8 of the treatment (the left side wound) **(personal photo 2023)**

- **Day 8 without the treatment (right side):**

-The untreated wound shows slow progress in closure, with the edges still having a significant gap.

-Granulation tissue continue to develop, but at a slower rate compared to the treated wound.

-The Inflammation persist, with redness and swelling still visible.



Figure 3.80 : Day 8 of the wound (without the treatment (the right side)(**personal photo2023)**

- **<u>Day 12 of the treatment (left side) :</u>**

-The wound exhibits significant progress in closure, with the edges further approximating.
-The granulation tissue becomes more prominent and bridges the wound gap.
-The Redness and swelling continue to diminish, approaching normal tissue appearance.



Figure 3.81: Day 12 of the treatment (left side) **(personal photo 2023)**

- **<u>Day 12 without the treatment (right side):</u>**

-The untreated wound still exhibit a noticeable gap between the edges, indicating slow closure.

-The Granulation tissue continues to develop, but the healing process remains slower compared to the treated wound.

-The Inflammation persist to some extent, with mild redness and swelling.



Figure 3.82: Day 12 without the treatment (right side) the forth rabbit **(personal photo 2023)**

- **<u>Day 15 of the treatment (the left side)</u> :**

The treatment was discontinued on day 12 because the wound had completely healed with a 100% recovery rate and total disappearance of any scar.

- **<u>Day 15 without the treatment (right side</u>) :**

After 15 days of the treatment in the other side and with complet healing 100 % we observed that the right side was in the beginning of the healing.



Figure 3.83: Day 15 of the treatment (the left side) **(personal photo 2023)**

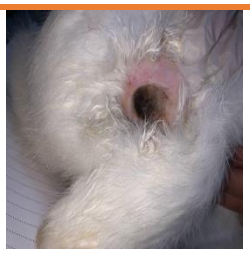
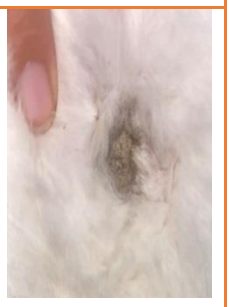
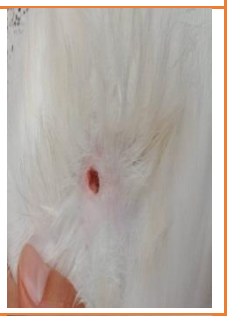
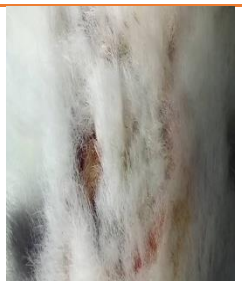
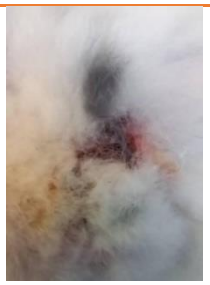


Figure 3.84: Day 15 without the treatment (the right side) **(personal photo 2023)**

3.8. The final result of the two creams (aloe vera pure gel and aloe vera with the active ingredient) in burns and wounds injury :

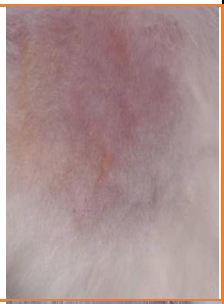
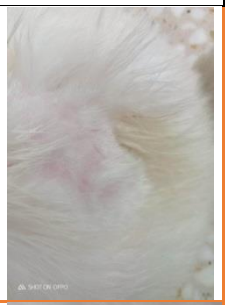
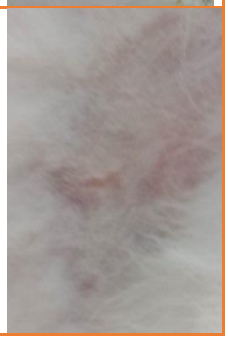
3.8.1. The Results of burns in the first and the third rabbits:

Table 3.5: The final result of the burns for 15 days in the both rabbits

		Day 1	Day 4	Day 8	Day12	Day 15
The first cream of aloe vera with pure gel	With the treat-ment					
	Witou ht the treatm ent					
The second cream Of aloe vera Active ingred ient	With the treat-ment					
	With out the treat ment					

3.8.2.<u>The Results of wounds in the second and the forth rabbits:</u>

Table3.6.: The final results of wounds in 15 days

		Day 1	Day 4	Day 8	Day12	Day 15
The first cream of aloe vera with pure gel	With the treat-ment					
	Witou ht the treatm ent					
The second cream Of aloe vera Active ingred ient	With the treat-ment					
	With out the treat ment					

Discussion

3.9.The plants extract yield :

In comparison between the three solvents used, aqueous and methanolic and hydroethanolic, it is observed that the aqueous solvent has a higher yield compared to the metnaolic and hydroethanolic solvent for the majority of the extracts from different plants.

The differences in yield can be attributed to various factors, including the solubility of bioactive compounds in different solvents, extraction conditions, and the nature of the plant material itself. Aqueous extraction is often used as a primary method due to its simplicity, cost-effectiveness, and the ability to extract a wide range of compounds. The high yield of the aqueous extract of *Moringa oleifera* and *artemisia* and *brocchia* suggests that the target compounds in this plant material are more soluble in water. Additionally, the specific composition of *Moringa oleifera*, such as the presence of water-soluble bioactive compounds, could contribute to the higher yield observed. On the other hand, the higher yield of the methanolic extract of *brocchia cenirea* leaves may indicate that the bioactive compounds in this plant material are better extracted using methanol as the solvent. Methanol is known to extract a diverse range of phytochemicals, including flavonoids, phenolics, and alkaloids, which could be present in *brocchia cenirea* leaves. These compounds may exhibit higher solubility in methanol compared to water, resulting in a higher yield.

3.10.Discussion of phytochemical screening :

Phytochemical screening is a valuable method used to identify and analyze the presence of various bioactive compounds in plants (falvinoids, anthocyans alkaloids,glugosids,quinons,mucilage,tannin) In this study, we conducted a comprehensive phytochemical screening of four plant species, specifically *Moringa oleifera, Artemisia herba alba , Brochia cenirea*, and *Aloe vera*. The leaf samples of these plants were utilized, while the gel of *Aloe vera* was subjected to analysis. By crushing the leaves into powder and extracting the gel from *Aloe vera.*

The present study reveals that the four plants (*Moringa oleifera, Artemisia herba alba , Brocchia cenirea, Aloe vera*) *shows* positive presence of flavinoids and mucilage ,*However,* the results also indicate that only three plants, specifically *Aloe vera, Brochia cenirea, and Artemisia herba alba,* display a positive presence of alkaloids. *Furthermore, the test for glucosides yielded* different results. It showed a positive presence only in *Brocchia cenirea and Artemisia herba alba , as well as Moringa oleifera.* Finally, tannins were identified in *Artemisia herba alba, Moringa oleifera, and Aloe vera.* this result can be attributed to their intrinsic biosynthetic pathways regulated by genetic constitution, physiological characteristics, and influenced by environmental factors such as light intensity, temperature, and nutrient availability. Stress conditions, including UV radiation or pathogen attacks, can stimulate an increased production of flavonoids as a defensive response. The specific types of flavonoids produced in each plant species may be influenced by their evolutionary adaptation to distinct ecological niches and selective pressures. It is important to note that the concentration and composition of flavonoids in these plants can vary based on factors such as genetic variations, growth conditions, and the specific plant part analyzed. Flavonoids in these plants have diverse biological roles and contribute to potential health benefits and therapeutic properties associated

with their consumption or utilization**. (Patel, P and all 2014/** *Benzidia, B.and all 2019/* **Dif, M. M and all 2018/ Chlif, Nand all 2022)**

In contrast to the presence of other compounds, the analysis revealed the absence of quinones and anthocyanins in all four plant species. ***Moringa oleifera*** **was found to lack alkaloids, while** ***Aloe vera*** **did not contain glucosides. Additionally, the tannin test revealed the absence of tannins in** ***Brocchia cenirea* ,** this result suggests that they do not produce these pigments. And it can be attributed to a combination of genetic factors, physiological characteristics, ecological adaptations, and environmental influences.

Genetic factors play a crucial role in determining the biochemical pathways and compound synthesis in different plant species. It is plausible that *Moringa oleifera, Aloe vera, Artemisia herba alba,* and *Brochia cenirea* lack the genetic components necessary for the production of quinones and anthocyanins.

Physiological characteristics also contribute to the absence of these compounds. Each plant species has its own metabolic priorities, which may favor the production of other compounds over quinones and anthocyanins.

The ecological adaptations of these plants to specific environments further contribute to the absence of quinones and anthocyanins. These plants have evolved in different ecological niches, facing distinct selective pressures. The absence of these compounds may provide them with advantageous traits for survival and reproduction in their respective habitats.

Environmental factors, such as light intensity, temperature, and nutrient availability, also influence the synthesis and accumulation of quinones and anthocyanins. The growth conditions and environmental characteristics of the habitats where Moringa oleifera, Aloe vera, Artemisia herba alba, and Brochia cenirea thrive may not favor the production or accumulation of these specific compounds.

It is important to recognize that the absence of quinones and anthocyanins in these plants does not diminish their overall significance. These plants possess their own unique composition of phytochemicals that contribute to their therapeutic properties and biological activities. Despite the absence of these particular compounds, they may still offer valuable health benefits and ecological functions based on their distinct biochemical profiles.

3.11.Discussion of antimicrobial activity :

This study aimed to evaluate the antifungal and antibacterial activities of three extracts (methanolic, hydroethanolic, aqueous) from four plants *(Moringa oleifera, Brocchia cinerea, Aloe vera, Artemisia herba alba*) and the essential oil of two plants (*Brocchia cinerea, Artemisia herba alba*) on the growth of seven bacterial strains (*S. aureus, P. aeruginosa, E. coli, Salmonella, Aspergillus, Candida albicans).*

The absence of antifungal activity in all extracts (methanolic, hydroethanolic, aqueous) for Artemisia herba alba and the aqueous extract of Moringa oleifera against the tested strains (*Candida albicans, Aspergillus brasiliensis*) could be explained by the absence of substances with antifungal activity in the Artemisia plant (**Bruneton, 1999**). and the difference between the compounds in the aqueous extract and the hydroethanolic and methanolic extracts for Moringa

oleifera. In fact, when transitioning from the aqueous total extract to the ethanolic extract, certain chemical groups are eliminated while others are concentrated (**Traoré, Y. & all,2012).**

The presence of antifungal activity in all extracts (methanolic/aqueous/hydroethanolic) of Brocchia cinerea and the hydroethanolic extract of Aloe vera can be explained by the presence of substances with antifungal activity in these plants, such as polyphenols, alkaloids, or flavonoids, which can have inhibitory effects on fungal growth. Additionally, certain extracts may act by modifying the structure and function of the fungal cell membrane.

- **Antimicrobial effect:**
- **Plants extracts:**

The difference in sensitivity observed among the 5 strains towards the same extract can be explained by the fact that the cell wall of Gram-negative bacteria contains a lipid layer that makes them less permeable and therefore more resistant compared to Gram-positive bacteria (**Mamadou, R.& all,2014).**

- **Positive results:**

The presence of antibacterial activity in certain extracts and all the essential oil can be explained by the presence of antibacterial compounds. Some plant extracts contain natural chemical compounds such as terpenes, phenols, flavonoids, alkaloids, and tannins that can have antibacterial properties. These compounds can act by disrupting the bacterial cell membrane. Furthermore, certain extracts can alter the structure and function of the bacterial cell membrane (**Mamadou, R.& all,2014).** After comparison of various concentrations within each extract it was found that 400 mg/ml is the optimal dilution concentration.

- **Negative results:**

The absence of antibacterial activity in certain extracts can be explained by the absence of antibacterial substances in the chemical composition of the plants. Additionally, it is possible that the concentration of the tested extract is insufficient to demonstrate significant antibacterial activity.

- **Aloe vera pure gel:**
- **Positive results :**

Pure aloe vera gel exhibits antibacterial activity against bacterial strains B. subtilis and S. aureus. This activity can be attributed to several factors and active compounds present in aloe vera.

Among these active compounds, anthraquinones, polysaccharides, and phytosterols can be found. Anthraquinones are natural compounds present in aloe vera, such as aloe-emodin and aloin, which have demonstrated antibacterial properties. These compounds can inhibit bacterial growth by disrupting essential cellular processes necessary for their survival.

Polysaccharides, such as acemannan, are also present in aloe vera and have shown antibacterial properties. These polysaccharides can inhibit bacterial growth by interfering with the metabolic processes of the bacteria.

Furthermore, phytosterols present in aloe vera, such as campesterol and β-sitosterol, can also contribute to its antibacterial activity. These compounds can damage bacterial cell membranes, leading to a loss of membrane integrity and inhibition of bacterial growth.

- **Negative results:**

The absence of antibacterial activity in pure gel of aloe vera can be explained by the absence of antibacterial substances in the pure gel or the resistant of the strains to aloe vera pure gel.

- **Diluted pure gel**:

The diluted pure aloe vera gel possesses the same antibacterial activities as the undiluted pure gel. This means that dilution can preserve the same properties and active components of the pure gel. It is interesting to note that the diluted gel demonstrates a higher activity than the undiluted gel. This improvement can be explained by the use of DMSO as a solvent, which promotes the penetration of the active compounds in aloe vera, thereby enhancing their effectiveness against bacteria.

- **Essential oil:**
- ***Artemisia herba alba :***

The essential oil of Artemisia herba-alba is known for its antibacterial activity. This activity is attributed to its specific chemical composition, which includes various volatile compounds such as terpenes, phenols, ketones, and aldehydes. These compounds play a crucial role in the pharmacological properties of the essential oil.

Among the active compounds present in the essential oil of Artemisia herba-alba, some have been identified as responsible for its antibacterial activity. These compounds include camphor, 1,8-cineole, camphene, artemetin, and other constituents found in the plant. These compounds can act in different ways to exert their antibacterial effect ***(Feng, X.& all,2020).***

Firstly, these compounds can interact with the cell membranes of bacteria. They can disrupt the membrane structure and cause leakage of essential substances, which can compromise the integriy of the bacterial cell and inhibit its growth.

Secondly, some compounds can interfere with the metabolic functions of bacteria. For example, they can inhibit key enzymes involved in vital metabolic processes for bacterial survival, leading to inhibition of their growth and multiplication.

- ***Brocchia cinerea:***

Our Studies have shown that the essential oil of brocchia cinerea has potentially interesting antibacterial activity. This antibacterial activity of the EO may be explained by its richness in oxygenated monoterpenes such as trans-Thujone, Camphor well known for their high and broadspectrum antimicrobial activity with the appreciable amount of 1,8-Cineol could also contribute to the antibacterial activity ***(Cheriti, A., & Boukhobza, Z,2020).***

3.12.Comparison between the antibiotique and the plant extracts and essential oils :

Compared to the antibiotics Amoxicillin, Aztreonam, and Streptomycin, *Artemisia Herba Alba* oil has shown superior results in most cases, with higher diameters of zone of inhibition.

This suggests a more pronounced antimicrobial activity of *Artemisia* oil against the different tested bacterial strains. The tested extracts, including *Brocchia cinerea* oil, demonstrated antimicrobial activity similar to that of antibiotics against the bacterial strains, except for the methanolic, hydroethanolic, and aqueous extracts of *moringa*, which showed considerably weaker activity compared to the antibiotics. The results suggest that the extracts have the potential to inhibit bacterial growth in a similar manner to conventional antibiotics. This indicates that the compounds present in these extracts could have beneficial antimicrobial properties. The obtained results suggest that the extracts have the potential to inhibit bacterial growth similarly to conventional antibiotics. This indicates that the compounds present in these extracts could have beneficial antimicrobial properties .

3.14.Discussion of The application of the two creams (pure gel)(active ingredient) on the the burns and the wounds

4.14.1Burns discussion:

In this study, two different degrees of burns were examinedand two different types of wounds (superficial wound and deep wound) . the effects of aloe vera cream were evaluated (two different creams were used) The aim of the study was to assess the potential benefits of using aloe vera in treating burns and wounds. two rabbits (superficial wound and third degree burn) were then treated ,we observed and documented the outcomes of this treatment.

The results of the study indicated a positive impact of applying two creams, namely pure gel and active ingredient, on both third and second degree burns as well as superficial and deep wounds.

After four days of treatment both rabbit's, the application of the two creams of *aloe vera* did not yield visible improvements on burns of both degrees(second degree and third degree *),*There were no signs of healing or tissue regeneration, Comparison with the untreated side showed similar characteristics, indicating that the aloe vera cream did not provide any noticeable advantages over natural healing in the first four days of the treatment .this results explained by Several factors may contribute to the lack of significant burn healing observed in the first four days of using aloe vera cream:

Firstly, the time frame of four days may be relatively short to expect noticeable healing, particularly for more severe burns. The intricate process of wound healing requires time, and initial stages often involve underlying processes that may not be immediately visible. The severity of the burn itself plays a vital role in the healing process. If the burn is deep or extensive, it may necessitate an extended period for noticeable improvements. Aloe vera cream may require more time to demonstrate its effects on such severe burns, as the healing process can be more complex. It can be the Individual variation also plays a significant role in treatment response. Each body and healing capacity are unique, influenced by factors such as skin type, overall health, and individual healing patterns. It is plausible that individual variations in response to the treatment contributed to the initial lack of observable healing. And maybe The application method and frequency of the aloe vera cream could also impact its effectiveness. Consistent and proper application is crucial for optimal results. Inadequate or inconsistent application of the cream may limit its ability to facilitate healing , Additionally, external factors

can influence the healing process. Underlying medical conditions, a compromised immune system, or the presence of infection can impede the healing process and potentially affect the effectiveness of the aloe vera cream.

The final results obtained of the treatment and the comparison with the untreated side (right side) provide valuable insights into the effects of aloe vera cream on the rabbit's burn wound . This suggests an improvement in the healing process and a reduction in the severity of the burn..It also showed resulted in blistering and the formation of fluid-filled sacs , This suggests that the cream stimulated a response in the skin, leading to the accumulation of fluids as part of the healing process. Blistering can be viewed as a positive sign, indicating that the aloe vera cream effort to protect and repair the damaged skin., The formation of a red scab on the rough-textured burned skin indicated the ongoing healing process, both of aloe vera creams helps to remove damaged cells and debris from the site of the burn, clearing the way for healing. And help to pull the burn edges closer together, minimizing the overall surface area of the burn.

In comparison, the untreated side of the rabbit's burn remained unchanged since Day 1, This results strongly suggests that the application of aloe vera cream on the treated side had a significant impact, manifesting in evident improvements and positive changes, which were clearly absent on the untreated side.

This result confirm that the Aloe vera has the abilities in promoting the healing of burns. When applied to burns, Firstly, the aloe vera gel like consistency provides a soothing and cooling sensation, effectively relieving pain and discomfort associated with burns. Additionally, aloe vera contains compounds such as polysaccharides and glycoproteins that possess anti-inflammatory properties. By reducing inflammation at the burn site, it aids in expediting the healing process. Furthermore, aloe vera acts as a natural moisturizer, preventing the drying out of burned skin and facilitating faster healing. The plant's bioactive compounds, including vitamins, minerals, and amino acids, are also believed to contribute to wound healing by stimulating collagen production, which is crucial for the regeneration of new skin tissue. Moreover, aloe vera exhibits antibacterial and antifungal properties, serving as a protective barrier against infections that can occur in burn wounds. aloe vera creates a more sterile environment for healing to occur.

3.14.2.Wounds injury discussion :

The results in the 15days period of the treatment with *Aloe vera* cream on the left side of the wound and the absence of treatment on the right side provide interesting insights into the effects of *Aloe Vera* on wound healing .

On the treated side (left), positive changes were observed after 15days of applying the cream. The cream has potential beneficial effects in wound healing, reducing inflammation, and promoting the formation of granulation tissue.It possesses anti-inflammatory properties and potential wound healing benefits

In contrast, on the untreated side (right), the wound injury remained largely unchanged in this period without any treatment.No significant signs of healing or tissue regeneration were observed, suggesting a lack of progress in the wound healing process .

This results confirm that the *Aloe vera* has captured attention for its remarkable potential in facilitating the healing of wounds, and its efficacy stems from a multitude of factors. The

presence of anti-inflammatory compounds such as bradykinase, salicylates, and polysaccharides within aloe vera plays a pivotal role in diminishing inflammation by impeding the release of inflammatory mediators and enzymes. Moreover, aloe vera boasts natural antimicrobial properties courtesy of its anthraquinones, lectins, and saponins, which exhibit impressive antibacterial, antiviral, and antifungal effects. By curbing the growth of microorganisms, aloe vera fosters a clean and protected environment for wounds, minimizing the likelihood of infections while supporting the healing process. Acting as a splendid natural moisturizer, aloe vera gel, abundant in water content, keeps wounds adequately hydrated, effectively averting excessive dryness and encouraging optimal wound healing. Furthermore, aloe vera possesses the extraordinary ability to stimulate collagen production, an indispensable protein fundamental to wound healing. Laden with essential amino acids, vitamins, and minerals, aloe vera bolsters collagen synthesis and remodeling, reinforcing structural integrity within the wound site and facilitating the formation of fresh tissue, thereby hastening wound closure and minimizing scar formation. Lastly, owing to its abundant reserves of polyphenols, flavonoids, and vitamins C and E, *aloe vera* exhibits antioxidant properties that successfully combat detrimental free radicals capable of impeding the healing process. By mitigating oxidative stress, *aloe vera* creates an environment conducive to optimal healing, accentuating its overall efficacy. These remarkable attributes collectively establish *aloe vera* as a wondrous natural remedy for wound healing, exemplifying the extraordinary potential harnessed within the confines of this remarkable plant.

Conclusion

In this study, we selected four plants: *Brocchia cinerea, Aloe vera, Artemisia herba alba*, and *Moringa oleifera*, with the aim of exploring new applications in the medical and pharmaceutical fields.

Our main objective was to extract essential oils from two plants, *Artemisia herba alba* and *Brocchia cinerea*, and prepare different types of extracts (methanolic, hydroethanolic, and aqueous) from the studied plants.

Additionally, we conducted a phytochemical screening of the studied plants to identify specific chemical compounds such as alkaloids, flavonoids, tannins, etc. Furthermore, we evaluated the biological properties of the plant extracts, focusing on their antibacterial activity, as well as the potential of hydroethanolic extract and pure Aloe vera gel to promote wound healing for burns and scars. To achieve this, we formulated two distinct creams: one based on the plant extract and the other based on pure Aloe vera gel. Subsequently, these creams were experimentally applied to burns and scars on rabbits to assess their effectiveness.

The extraction results demonstrated varying yields depending on the solvents used. The phytochemical screening revealed the presence of chemical compounds in all the plants, which could be responsible for the observed biological activities. Furthermore, the tested extracts and essential oils exhibited varying sensitivity against the studied strains, with inhibition zones ranging from 8.6 to 60.7 mm.

The results of the wound healing and burn tests, involving the application of dermatological creams, indicated that both creams showed excellent wound healing 5 superficial wound and deep wound) activity (100% efficacy for the pure gel-based cream) within a 12-day period. They also demonstrated significant effects on burns (3rd/2nd degree).

perspectives:

- ✓ Further investigate the biological activity by expanding the range of microbial strains studied.
- ✓ Identify the molecules responsible for the biological activities, determine the chemical structure of phenolic compounds, and assess the toxicity of the extracted molecules before using them in formulations.
- ✓ Conduct additional biological activities such as antioxidant and anti-inflammatory evaluations.
- ✓ Optimize the formulations by adjusting the concentrations of active ingredients and exploring new excipients.

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