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Antibacterial activities of phenolic extracts of Eriobotrya japonica lindl ; leaves ; stem and root.

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Dedication

We dedicate this thesis to our **dear parents**, the true pillars of our lives. Their unwavering love, endless patience, and silent sacrifices have been the foundation upon which we have built our dreams. Through their steadfast trust, constant support, and daily encouragement, we found the strength to overcome every obstacle. They have always guided us with wisdom and kindness, lighting our path even during the toughest times. To them, we express our deepest gratitude, profound respect, and eternal love. Without them, none of this would have been possible.

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Abstract

The emergence of antibiotic-resistant bacterial strains, particularly avian pathogenic *Escherichia coli* (APEC), represents a major challenge for poultry health and food safety. This work aims to evaluate the antibacterial activity of hydroalcoholic extracts obtained from the leaves, stems, and roots of *Eriobotrya japonica* Lindl.

The theoretical part covers the botanical, phytochemical, and pharmacological aspects of *Eriobotrya japonica* Lindl, phenolic compounds, and the characteristics of avian pathogenic *Escherichia coli* (APEC), including its pathogenic mechanisms, modes of transmission, and control strategies.

Our study first involved hydroalcoholic extraction using the Soxhlet method, analysis of phenolic content using the Folin-Ciocalteu method, and FTIR-ATR characterization. Secondly, we evaluated the antibacterial activity against several APEC strains using antibiogram, dilution method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests.

The results show analyses revealed a higher concentration of phenolic compounds in the leaves compared to the other parts of the plant, accompanied by moderate antibacterial activity against the tested strains (ATCC 25922, and 7 isolates). This antibacterial activity gradually decreases from leaves to stems and then to roots.

This plant may also have potential for the development of complementary or preventive treatments in humans, especially in response to the growing emergence of multidrug-resistant bacteria. However, further studies are needed to confirm its efficacy and safety for human medical use.

Keywords: *Eriobotrya japonica* Lindl*,* Avian pathogenic *Esherichia coli* (APEC), antibacterial activities, phenolic compounds, FTIR–ATR.

Résumé

L'émergence de souches bactériennes résistantes aux antibiotiques, notamment *Escherichia coli* pathogène aviaire (APEC), constitue un enjeu majeur pour la santé avicole et la sécurité sanitaire. Ce travail vise à évaluer l'activité antibactérienne des extraits hydroalcooliques issus des feuilles, tiges et racines d'*Eriobotrya japonica* Lindl.

La partie théorique aborde les aspects botaniques, phytochimiques et pharmacologiques de la plante *Eriobotrya japonica* lindl, les composés phénoliques, ainsi que les caractéristiques d'Escherichia coli aviaire pathogène (APEC), incluant ses mécanismes de pathogénicité, ses modes de transmission et les stratégies de contrôle.

Notre étude consiste en premier lieu l'extraction hydroalcoolique par la méthode du Soxhlet, l'analyse du contenu phénolique par la méthode de Folin-Ciocalteu, la caractérisation FTIR-ATR. On second, l'évaluation de l'activité antibactérienne contre plusieurs souches d'APEC par antibiogramme, dilution, CMI et CMB.

les résultats montrent les analyses ont révélé une concentration plus élevée en composés phénoliques dans les feuilles, comparativement aux autres parties de la plante , accompagnée d'une activité antibactérienne modérée contre les souches testées (ATCC 25922 et 7 isolats). Cette activité antibactérienne diminue progressivement des feuilles vers les tiges, puis vers les racines.

Cette plante pourrait également présenter un intérêt potentiel pour le développement de traitements complémentaires ou préventifs chez l'humain, notamment face à l'émergence croissante de bactéries multi résistantes. Des études supplémentaires restent toutefois nécessaires pour confirmer son efficacité et sa sécurité d'utilisation en médecine humaine.

Mots clés :*Eriobotrya japonica* Lindl *, Escherichia coli* pathogène aviaire (APEC), Activité antibactérienne , composés phénoliques ,FTIR –ATR .

الملخص

يمثل ظهور السلالات البكتيرية المقاومة للمضادات الحيوية، ولا سيما الإشريكية القولونية الممرضة للطيور، مشكلة كبيرة لصحة الدواجن وسلامتها. الهدف من هذه الدراسة هو تقييم النشاط المضاد للبكتيريا للمستخلصات المائية الكحولية المستخلصة من أوراق وسيقان وجذور نبات إريوبوتريا جابونيكا ليندل. ويغطي الجزء النظري الجوانب النباتية والكيميائية النباتية والدوائية لنبات الإريوبوتريا جابونيكا، والمركبات الفينولية، وخصائص الإشريكية القولونية الممرضة للطيور، بما في ذلك آليات إمراضها وطرق انتقالها واستراتيجيات مكافحتها.

تألفت دراستنا أولاً من الاستخلاص المائي الكحولي باستخدام طريقة سوكسليت، وتحليل المحتوى الفينولي باستخدام طريقة فولن-سيوكالتو، وتوصيف .FTIR-ATR ثانياً، تم تقييم النشاط المضاد للبكتيريا ضد العديد من سلالات APEC عن طريق المضادات الحيوية والتخفيف و MIC و.MBC أظهرت النتائج وجود محتوى مركب فينولي عالٍ، خاصة في الأوراق، مصحوباً بنشاط مضاد للبكتيريا معتدل ضد السلالات التي تم اختبارها ATCC 25922 و7 سلالات تضاءل هذا النشاط المضاد للبكتيريا تدريجياً من الأوراق إلى السيقان ثم إلى الجذور. كما يمكن أن تكون هذه النبتة ذات أهمية محتملة في تطوير علاجات تكميلية أو وقائية للبشر، خاصة في مواجهة الظهور المتزايد للبكتيريا متعددة المقاومة. ومع ذلك، لا تزال هناك حاجة إلى مزيد من الدراسات لتأكيد فعاليته وسلامته في الطب البشري.

الكلمات المفتاحية: إيريوبوتريا جابونيك, النشاط المضاد للبكتيريا, الإشريكية القولونية الممرضة للطيور ,المركبات الفينولية ,مطيافية الأشعة تحت الحمراء.

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Experimental part.

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List of Abbreviations

- **APEC** : Avian Pathogenic *Escherichia coli.*
- **ATCC** : American Type Culture Collection.
- **ATR** : Attenuated Total Reflectance.
- **BHI(B)** : Brain Heart Infusion (Broth).
- **CFU** : Colony-Forming Unit.
- **CLSI** : Clinical and Laboratory Standards Institute.
- **DMSO** : Dimethyl Sulfoxide.
- **ELISA** : Enzyme-Linked Immunosorbent Assay.
- **ESBL** : Extended-Spectrum Beta-Lactamase.
- **FTIR** : Fourier Transform Infrared Spectroscopy.
- **GAE** : Gallic Acid Equivalent.
- **HPLC** : High-Performance Liquid Chromatography.
- **MBC** : Minimum Bactericidal Concentration.
- **MIC** : Minimum Inhibitory Concentration.
- **OD** : Optical Density.
- **PCR** : Polymerase Chain Reaction.
- **rpm** : Revolutions per Minute.
- **UV** : Ultraviolet.

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General Introduction

In recent years, the search for natural bioactive compounds has gained increasing attention, particularly in the field of pharmacology and microbiology. With the emergence of antibiotic-resistant bacterial strains, the need for alternative antimicrobial agents has become an urgent priority in both human and veterinary medicine. Plants, as rich sources of secondary metabolites, offer a promising reservoir of such compounds, including phenolics, flavonoids, tannins, and other phytochemicals with known biological activities.

Among medicinal plants, *Eriobotrya japonica* Lindl (commonly known as the loquat) has attracted scientific interest due to its wide range of traditional uses and pharmacological properties. Native to southeastern Asia and cultivated in many parts of the world, *Eriobotrya japonica* Lindl has been used in folk medicine to treat respiratory, gastrointestinal, and inflammatory disorders. Its leaves, stems, and roots are known to contain significant levels of phenolic compounds, which are largely responsible for its antioxidant and antimicrobial activities.

Escherichia coli, particularly avian pathogenic strains (APEC), pose a serious threat in poultry farming, leading to severe economic losses and representing a potential risk to public health due to zoonotic transmission and antimicrobial resistance. Investigating the antibacterial potential of natural extracts against such pathogens is therefore of high relevance.

This study aims to evaluate the phenolic content of *Eriobotrya japonica* Lindl extracts obtained from different plant parts (leaves, stems, and roots) and to assess their in vitro antibacterial activity against multiple strains of APEC. Through phytochemical analysis, spectrophotometer FTIR-ATR spectroscopy, and microbiological assays such as microdilution methods.

This thesis is divided into two main parts: the theoretical part and the practical part. The theoretical part comprises three chapters. The first chapter focused on the plant *Eriobotrya japonica* Lindl, including its botanical description, uses, and other relevant information.

The second chapter discussed phenolic compounds, covering their chemical structure, classification and biosynthesis.

The third chapter addressed the *Avian Pathogenic Escherichia coli (APEC)*, its structure, characteristics, and other important features.

The practical part included the materials and methods used in this study. Extraction was performed using the Soxhlet method, analytical techniques such as Spectrophotometer FTIR-ATR spectroscopy and antibacterial activities assessments, including antibiogram, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC).

The final sections include the presentation and discussion of the experimental data, concluding with a general conclusion that reflects on the findings and their potential implications.

Theoretical part

Chapter I
Eriobotrya japonica Lindl.

I.1. History

Eriobotrya is a Greek name consisting of “erion” and “botrus” which means respectively wool and cluster, this genus includes about thirty shrubs or trees. Persistent, native to Asia for more than 2000 years. In France, it was only around 1831 that we began to consider the loquat as a fruit tree **(EL and *al,* 2015)**, then their cultivation was described in various countries such as Algeria, Egypt, Greece, Italy, Spain, Tunisia and Turkey **(En and *al*., 2016)**.

I.2. Generality

Eriobotrya japonica Lindl is a fruit-bearing tree from the Rosaceae family **(Dhiman and *al*., 2021)**. It is native to southeastern China and has been cultivated in Asia for centuries for its sweet and juicy fruits. The plant was introduced to Japan over a thousand years ago, where it became widely grown hence the common name "Japanese loquat." The species was first formally described by Swedish botanist Carl Peter Thunberg in the 18th century, who was among the first Europeans to classify many Asian plants **(Bostan and *al.,* 2018).**

I.3. Taxonomic

The first taxonomist to officially describe *Eriobotrya japonica* was the Swedish botanist Carl Peter Thunberg (1743-1828) **(Thunberg , 1784)**.

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Rosales
Family: Rosaceae
Genus: Eriobotrya
Species*: Eriobotrya japonica* (Thunb.) Lindl **(Lindley, 1821).**

I.4. Local names

The real Arabic name of the *Eriobotrya japonica* Lindl is El Bachmalat بشملة other names according to regions (M'chimcha in Algiers, El-molléce in Béchar and Oran, zâroura and bou-âdima in Tlemcen and Zaarour in Constantine ,denefle) **(En and *al*., 2016).** Chinese (luju, biba), English (loquat), Japanese (medlar), French (bibassier du Japon, bibace, néflier du Japon), German (Loquate, japanische mispel),

Hindi (lokat), Indonesian (papalaan, okwat), Italian (nespola Giapponese, nispolero), Portuguese (ameixa do Japao), Spanish (nespereira, níspero de Japón); Trade name (loquat) **(Janick and Paull, 2008).**

I.5. Botanical Description

Eriobotrya japonica Lindl is an evergreen shrub or small tree 6- 8 m high; bole usually rather short, 0.6-1 m long, surmounted by a dense ovoid or globular crown; bark grey and shallowly fissured, on young branches it is pale brown and hairy **(Fig1)**.

Figure 1: Tree of *Eriobotrya japonica* Lindl **(Morton, 1987).**

-The leaves are slightly crowded towards the end of the stem, large, alternating, subsessile, stiff, leathery, lanceolate in shape, 21-32 cm long, with margins slightly toothed to strongly toothed ; dark, green, narrowed into a very short petiole **(Fig 2)**.

Figure 2: Leaves of *Eriyobotrya japonica* Lindl **(Morton, 1987).**

-**Flowers** fragrant, 1.2 cm broad, borne in woolly panicles, 10-20 cm long; calyx composed of 5 small, imbricate, acute teeth; corolla has 5 oblong, ovate-clawed petals, white in colour and delicate in texture; stamens 20; pistils 5 (**Janick and Paull, 2020) (Fig 3).**

Figure 3: Flowers of *Eriobotrya japonica* Lindl **(Cistus Nursery 2025).**

-**Fruit** in clusters, generally round, oval or pyrethral, 2.5-8 cm long, pale yellow to orange, slightly mellow on the surface; skin thick like a peach but slightly harder; flesh firm and fleshy in some varieties; colour ranging from near white to dark orange; Juicy and subacid; 4-10 seeds, 1-2 cm long **(Janick and Paull 2020) (Fig 4).**

Figure 4: Fruit of *Eriobotrya japonica* Lindl **(Morton. (1987).**

-Roots Generally, have a taproot system (**Gilman and Dennis, 2022) (Fig5).**

Figure 5: root of *Eriobotrya japonica* Lindl (Greer 2025).

I.6. Reproductive Strategy

Hermaphroditic species, the self-incompatibility of *E. japonica* is gametophytic. Cloned trees flower readily within 1-2 years, but worthwhile fruit set takes a few more years. Honeybees are its pollen vectors. After fertilization, the fruit develops very rapidly, birds and bats disperse the fruit **(Freihat and *al., 2008).***

I.7. Ecology and biophysical limits

Originally from regions with a subtropical climate, *E. japonica* requires a mild climate with rainfall evenly spread throughout the year, without excessive heat, especially during fruit ripening. The most favourable conditions for productivity and quality are to be found near the Once established, it is tolerant of drought and of slight frost (**Yukio Nagano and *al.,* 2022)** .Temperatures lower than -5°C damage the flowers, and those lower than -12 ° C are fatal. Biophysically, the species thrives at altitudes between 700 and 2400 meters, in areas with an average annual rainfall ranging from 650 to 1000 mm. It can grow on a wide variety of soil types from sands to heavy clays but prefers acidic soils over alkaline ones **(Fernández-López, and Gil-Sánchez., 2021).** Optimal growth occurs in light, deep, well-drained, and moist alluvial soils, particularly those with a gritty subsoil extending to a depth of approximately 1.5 meters **(Nagao and Matsuo, 2008).**

I.8. Species distribution in the world

The map above shows countries where the species has been planted. It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site **(Infante-Rodríguez and *al*., 2024).** Exotic range Native range China, Japan Albania, Algeria, Argentina, Australia, Brazil, Cambodia, Chile, Colombia, Cyprus, Ecuador, Egypt, Eritrea, Ethiopia, France, French Guiana, Germany, Greece, Guatemala, Guyana, Honduras, India, Indonesia, Italy, Kenya, Laos, Libyan Arab Jamahiriya, Madagascar, Malaysia, Malta, Mexico, Morocco, Myanmar, Nicaragua, Panama, Philippines, Portugal, South Africa, Spain, Surinam, Taiwan, Province of China, Tanzania, Thailand, Trinidad and Tobago, Turkey, Uganda, United Kingdom, United States of America, Venezuela, Vietnam **(Crane and Caldeira., 2019) .**

The map above shows countries where the species has been planted. It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, you need to follow biosafety procedures that apply to your planting site **(Fig 6) (Orwa *and al*., 2009).**

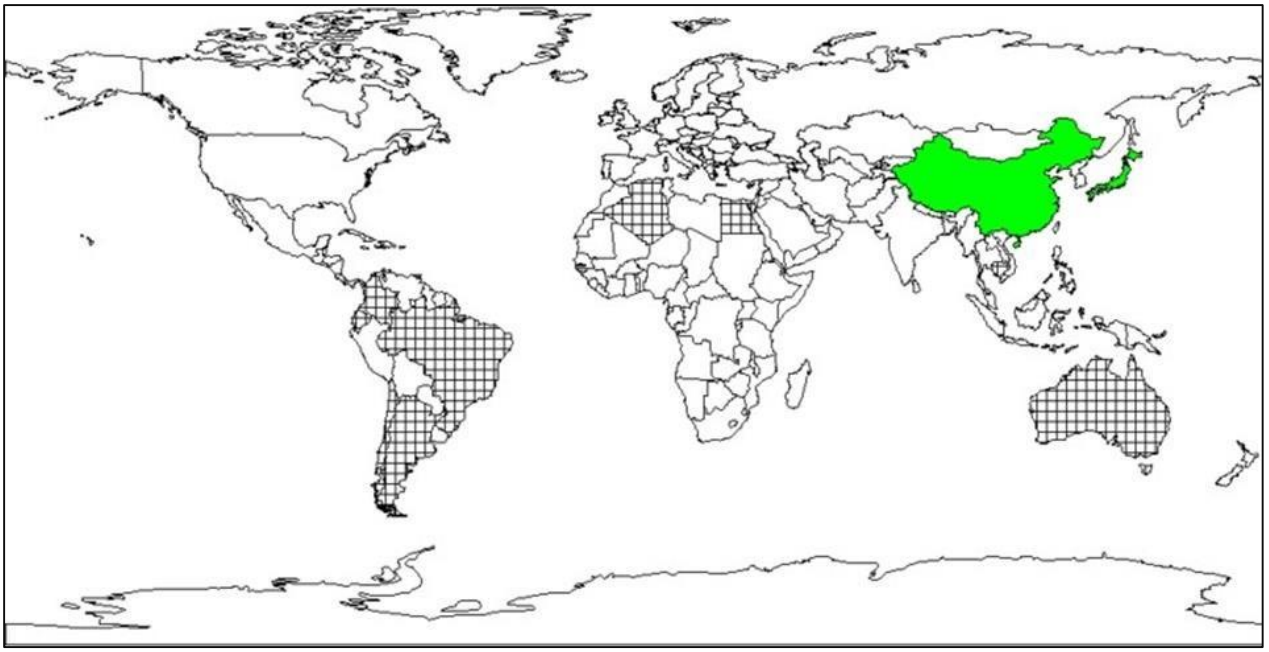


Figure 6: Geographical distribution of *Eriobotrya japonica* Lindl **(Orwa and *al*., 2009).**

Native range China, Japan.

Exotic range.

I.9. Utilisations

Eriobotrya japonica Lindl has been included in "The Plant List" and it is the most widely researched species in its genus. Its dried leaves are widely used in traditional Chinese herbal medicine to treat coughing caused by pulmonary inflammation, dyspnea due to asthma and cough, nausea caused by stomach disorders, restlessness, and thirst. Furthermore, it is used to treat stomach ache, ulcers, chronic bronchitis, cancer, and diabetes mellitus in Japanese folk medicine **(Zhu and Jiang., 2022).**

Triterpenes and flavonoids are the most important bioactive compounds responsible for pharmacological activities, such as anti-inflammatory, and antitumor activities. Other beneficial physiological effects such as antioxidant, hepatoprotective, bronchodilatory and expectorant effects and tracheal smooth muscle relaxation.

High doses (2.000 mg/kg) of *E. japonica* leaf extracts have been used in laboratory animals, and no side efects or toxicity symptoms have been observed **(Xu zhu and *al*., 2022).**

I.10. Pharmacological activities.

I.10.1. Anti-inflammatory Activities

Extracts from loquat leaves have shown significant anti-inflammatory effects by inhibiting inflammatory mediators such as nitric oxide, prostaglandins, and cytokines. This makes it potentially useful for managing chronic inflammatory conditions..Pharmacological Effects of *Eriobotrya japonica* Lindl **(Kim and *al.,* 2010).**

I.10.2. Hepatoprotective Effects

The plant extracts may protect liver cells from damage caused by toxins, oxidative stress, or drugs, aiding liver health **(Alqasoumi and *al*., 2018).**

I.10.3. Antioxidant Properties

Eriobotrya japonica Lindl exhibits strong antioxidant activity, mainly due to its leaves, which are rich in phenolic compounds and flavonoids. These bioactive substances help neutralize free radicals, protecting cells from oxidative stress. Leaf extracts have shown significant antioxidant potential in various studies. **(Zhang and *al*., 2016).**

I.11. Phytochemical Composition

Eriobotrya japonica Lindl, commonly known as loquat, is a traditional medicinal plant rich in various bioactive phytochemicals found in its leaves, fruits, and seeds. These constituents are responsible for its diverse pharmacological properties **(Zhou and *al*., 2011).**

I.11.1. Flavonoids

The leaves of *E. japonica* are particularly rich in flavonoids, which are known for their strong antioxidant and anti-inflammatory properties **(He and *al*., 2015).**

I.11.2. Triterpenes and Triterpenoids

These pentacyclic compounds are abundant in the leaves and contribute to various therapeutic effects **(Yuan and Xu, 2012).**

I.11.3. Phenolic Acids

Several phenolic acids have been identified in the plant extracts **(Barbi and *al*., 2022).**

I.11.4. Tannins

The plant contains hydrolyzable tannins, such as gallotannins and ellagitannins, which have antioxidant, antimicrobial, and gastrointestinal protective effects **(Yuan and Xu, 2012).**

I.11.5 alkaloids

Alkaloids in *Eriobotrya japonica* Lindl could have important bioactive roles. Detected in leaves and seeds, they may enhance the plant's pharmacological profile when combined with other secondary metabolites **(Uddin and Rehman .2012).**

Chapter II
Phenolic compounds

II.1. Generality

Compounds with more or single aromatic rings coupled to a single or more hydroxyl groups are commonly called phenolic. They are the most common secondary plant metabolites with over 8000 known structures. They range from the simple phenolic such as phenolic acids; to the complex compounds like tannins.

The compounds participate in plant defence against ultra violet (UV), pathogens, and other predators **(Kiselev and Dubrovina, 2023)**. Their presence in all plant organs makes them a vital ingredient of the human diet. Phenolics are found mainly in fruits, legumes, vegetables, tea, wine, coffee, and accounts for the organoleptic characteristics of plant food **(Zhang and *al.,* 2024)**.

Likewise, phenolic compounds are responsible for the bitterness of fruits due to their interaction with salivary glycoprotein. Phenolics can also added to the colour of many fruits and vegetables. Among the plants, phenolics are lignans, tannins, phenolics acids, stilbenes, and flavonoids **(Oluwaseun and *al.*, 2021).**

II.2. Phenolic compounds from natural sources

Plants can synthesize several organic compounds called secondary metabolites either during normal metabolic processes or in regarding to certain environmental conditions including wounds, temperature, UV-radiation, infection, and others **(Kumar and *al.*, 2024)**. These metabolites are grouped into different clusters in relative to the occurrence of phenol rings in their structures, and on the structures that hold the ring in place. Phenolic compounds occurred as functional derivatives such as methyl esters, esters and glycoside **(Shahidi and Yeo, 2018)**.

They are seen inform of the conjugate with poly- and monosaccharides which joined one or more phenolic compounds. They include flavonoids, phenolic acids, simple phenols, and hydroxycinnamic acid derivatives.

Phenolic compounds are categorized into different classes**.** They are toxic to micro-organisms due to the presence of several numbers of hydroxyl groups on the phenols **(Vermerris and *al.*, 2009)**. Phenol (also known as carbolic acid, phenolic acid, or benzenol) is an aromatic organic compound with the molecular formula C6H5OH It is a white crystalline solid that is volatile. The molecule consists of a phenyl group (−C6H5) bonded to a hydroxy group (−OH) **(Gośliński and *al.*, 2021) (Fig 7).**

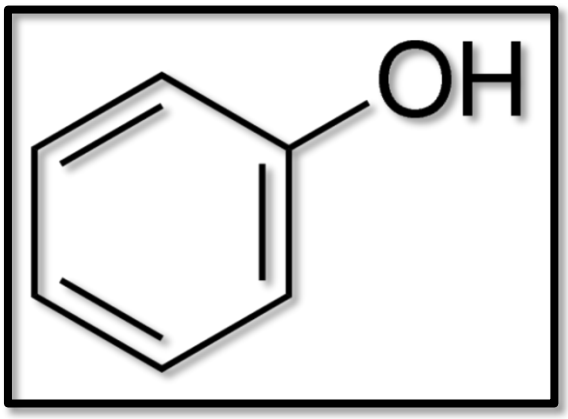


Figure 7: Structure of phenol. **(Smith, 2011).**

II.3. Classification

Phenolic compounds can generally be classified into simple and polyphenolic compounds **(Vermerris and *al*., 2009) (Fig 8).**

II.3.1. Simple phenolic compounds

Phenolic compounds that contain one phenol unit (or a derivative of it) are considered "simple". Fundamentally, they are substituted phenol compounds. Simple phenolic compounds have C6 general skeleton representation. The general structure is shown below. The group denoted by "R" (an organic group which could be alkyl, alkenyl,.etc. **(El-Sayed, 2021)**. or hydroxy, amino ...etc.) which can be in the ortho (o), meta (m), or para (p) positions of the aromatic ring. These descriptors refer, with respect to the position of the hydroxyl group constituting phenol which is given position 1, to 1,2-, 1,3, and 1,4-carbon relationship respectively. Below are some simple phenolic compounds **(Vauzour, 2014) (Fig8).**

II.3.1.1. Phenolic acids

Phenols that contain a carboxylic acid are termed as phenolic acids. If the carboxylic acid functional group is directly bonded to the phenol ring, the phenolic compound is termed as hydroxybenzoic acid. When carboxylic acid functional group and the phenol ring are separated by two doubly bonded carbons (a C=C bond), phenolic compounds are termed as hydroxycinnamic acids **(Khater, 2011) (Fig8).**

II.3.1.1.1. Hydroxybenzoic acids

Hydroxybenzoic acids are benzoic acids substituted with a hydroxyl group. Alternatively, they can be viewed as phenols that are substituted with a carboxylic acid functional group that is directly bonded to the phenol ring

(Gomes da Silva and *al.*, 2023). The hydroxyl group in hydroxybenzoic acids can be ortho (o) (salicylic acid), meta (m), or para (p). The structures are shown below. Dihdyroxybenzoic acids are benzoic acids that are substituted with two hydroxyl groups. The two hydroxyl groups can mainly be in 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5 relative positions. Trihdyroxybenzoic acids are benzoic acids that are substituted with three hydroxyl groups. Examples include 2,4,6-trihydroxybenzoic acid and 3,4,5-trihydroxybenzoic acid (gallic acid) **(Das and *al*., 2019) (Fig8).**

II.3.1.1.2. Hydroxycinnamic acids

When the carboxylic acid functional group is separated from the phenol ring by a C=C bond, phenolic acids are described as hydroxycinnamic acids. Examples of hydroxycinnamic acids are 2-, 3-, and 4-hydroxycinnmaic acid shown below. Other common examples of cinnmaic acids are caffeic acid, ferulic acid, and sinapic acids shown below **(Hassaine, 2020) (Fig8).**

II.3.1.2. Coumarins

Hydroxycoumarins are hydroxyl-substituted coumarins. They are examples of phenolic compounds**.** The first coumarin was isolated from the tonka bean (Coumarina odorata). The cyclization of hydroxycinnamic acid leads to the formation of coumarins. They are widespread, especially among dicotyledons, particularly in the roots and bark **(Aitor and *al*., 2021) (Fig8).**

II.3.2. Polyphenols

Phenolic compounds that contain more than one phenol unit are considered “polyphenol”. Polyphenolic compounds have C15 general skeleton representation **(Belščak-Cvitanović and *al.*, 2018) (Fig8).**

II.3.2.1. Flavonoids

Flavonoids are polyphenolic compounds with the general structure shown below. Generally, rings A and C are either mono, di, or trihydroxylated. The O-heterocycle B is usually a pyrone ring as in Luteolin but could also be a pyrlium ring as in delphinidin. If ring C is attached to C2 of ring B, the flavonoid is a flavone (as Luteolin), flavonol (as kaempferol), an anthocyanin (as delphin-din) or a flavanone (as naringenin) **(Zhuang and *al.*,2023)**. If the ring C is attached to C3 of ring B, then the flavonoid is an isoflavone such as daidzein. Chalcones such as chalcone, are a class of flavonoids in which rings A and C are separated by 3-carbon linear chain rather than a ring. The bond between C2 and C3 of ring B is commonly double as in flavones, flavonols,

chalcones and isoflavones. However, the C2-C3 bond could be single as in flavanones **(Ghnimi, 2015) (Fig8).**

II.3.2.2. Tannins

Tannins are known to bind to and precipitate proteins and amino acids. They are subdivided into three types; hydrolyzable, condensed and complex. Hydrolyzable tannins can be gallotannins or ellagitannins. Gallotannins are polyols that are substituted with gallic acid units. The galloyl units in gallotannins are linked by depside (ester) linkages. Commonly the polyol core is a D-glucose that is substituted with gallic acid units **(He and.,*al* 2022)**. Tannic acid is an example of gallotannins. Similar to gallotannins, ellagitannins are hydrolysable 1,2,3,4,6-pentagalloyl-glucose. However, unlike gallotannins characterized by depside linkages, adjacent galloyl groups in ellagitannins are linked by C-C bonds. Condensed tannins are polymeric phenolic compounds that consist of catechin units. When depolymerized, they give anthocyanidin. Thus condensed tannins are called proanthocyanidins. Complex tannins are gallotannins or ellagitannins bonded to a catechin unit **(Rira, 2019) (Fig8).**

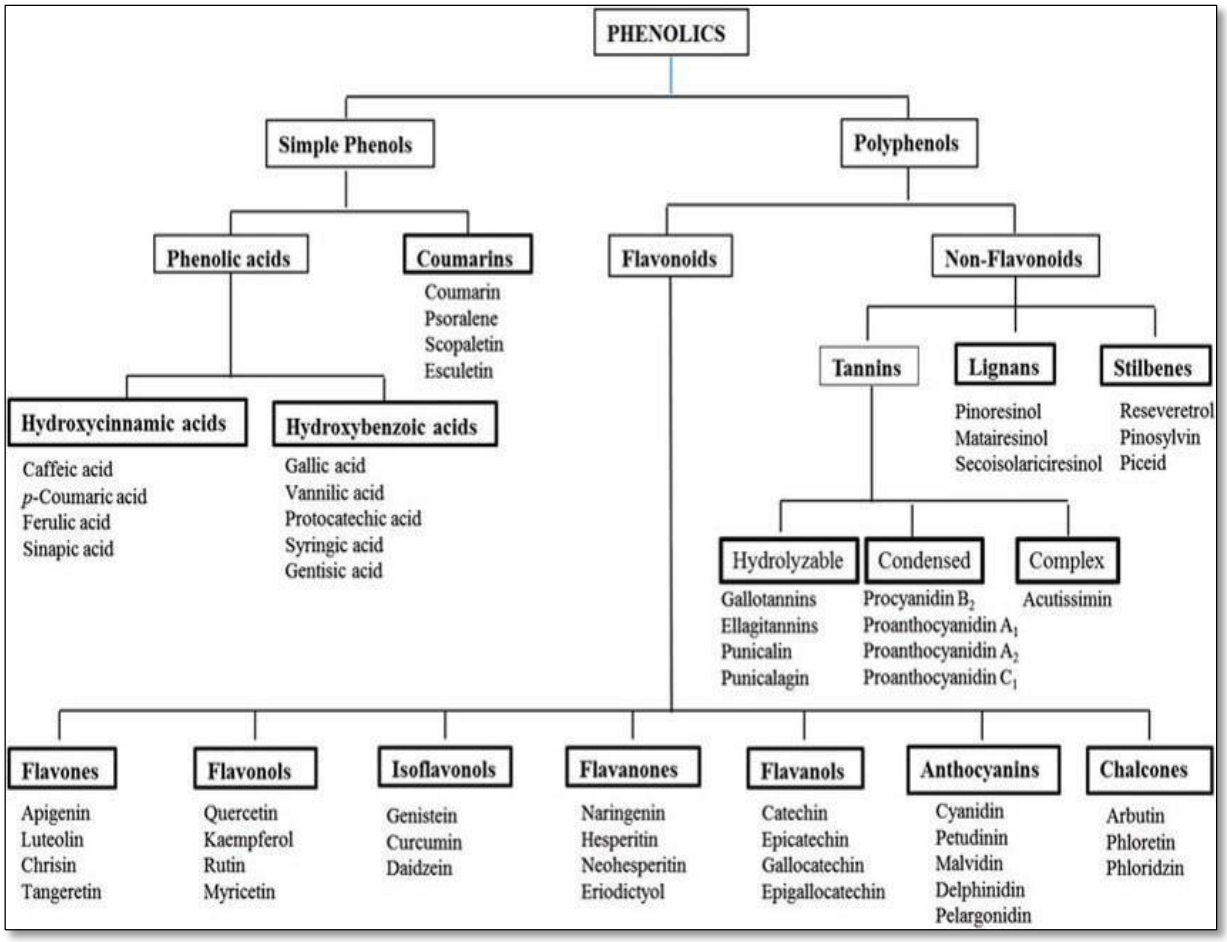


Figure 8: Different classes of phenolic compounds **(Vermerris and *al.*, 2009).**

II.4. Biosynthesis

There are two general routes for the biosynthesis of phenolic compounds; shikimic acid pathway and the acetic acid pathway. **(Marchica and *al.*, 2020)** In the shikimic acid pathway, hosphoenolpyruvate and erthrose-4-phosphate react in few steps to provide 3-dehydroquinate. Dehydration with shikimate dehydrogenase gives 3-dehydroshikimic acid. Reduction with NADPH gives shikimic acid. 3-Dehydroshikimic acid could lead to gallic acid in several steps. Shikimic acid is then converted into chorismic acid which undergoes Claisen rearrangement to afford prephenic acid. The product is then converted in several steps into tyrosine **(Shende and *al.*, 2024)**. The amino acid serves as a central point and a crucial precursor for the biosynthesis of various phenolic compounds. Another route toward phenolic compounds, is the phenylpropanoid pathway. This route is essentially similar to the shikimic acid pathway until L-phenylalanine stage where the phenylpropanoid pathway takes form. L-Phenylalanine undergoes deamination catalyzed by phenylalanine ammonia lyase (PAL) enzyme to give cinnamic acid. Hydroxylation followed by conversion to the Coenzyme A provides p-coumaroyl Coenzyme A. This molecule serves as a central point toward various phenolic compounds **(Ibrahim and *al*., 2020) (Fig 9).**

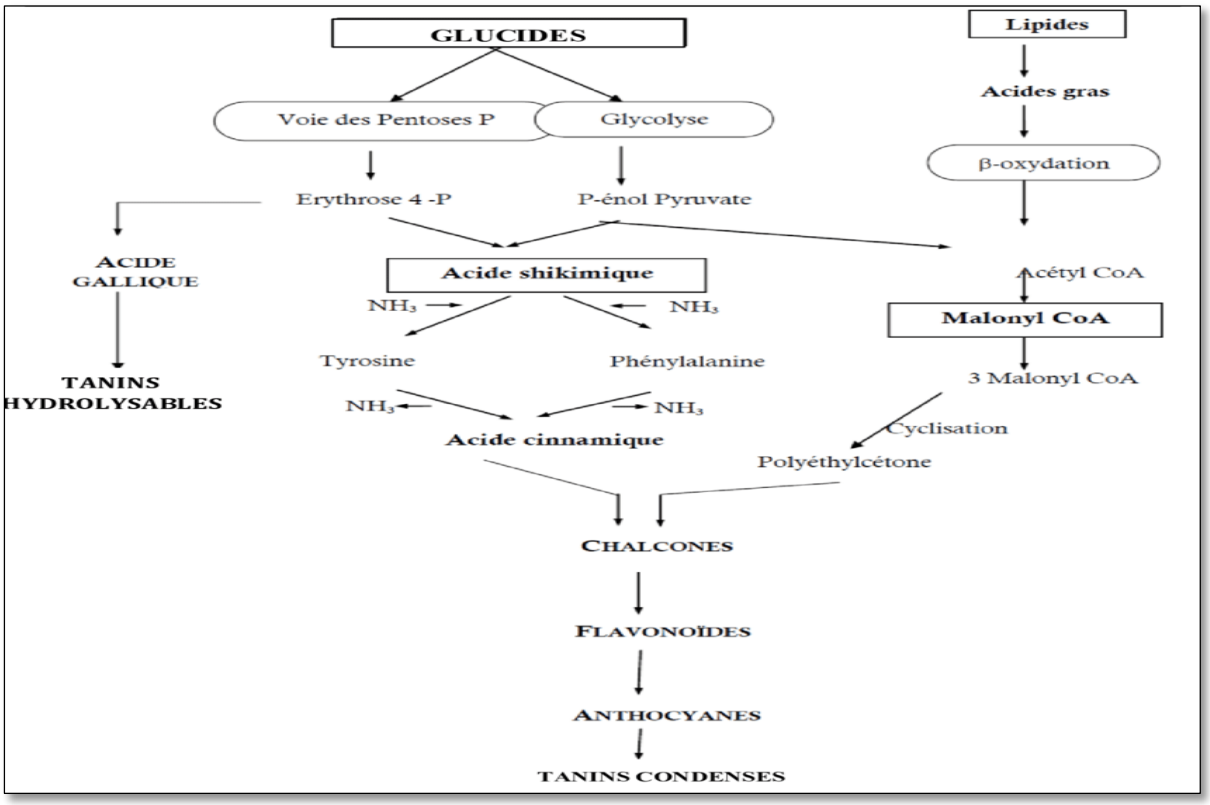


Figure 9: The biosynthesis pathways of phenolic compounds **(Zhang and *al*., 2012).**

II.5. Utilisations

II.5.1. Health and Disease

In pharmacology, phenolic compounds are actively studied for their role in modulating various therapeutic pathways. They act as anti-inflammatory, antimicrobial, antioxidant, and anticancer agents in numerous experimental models **(Zhang and Zhang, 2023)**. Several natural or semi-synthetic drugs are now derived from or inspired by phenolic structures due to their ability to target key enzymes, cellular receptors, or signaling pathways involved in chronic diseases **(Zhou and Zheng, 2016).** Their integration into modern pharmacological strategies reflects their therapeutic potential, particularly as adjuvants or alternatives to conventional treatments, especially in response to rising microbial resistance and the side effects of synthetic drugs **(Kumar and Pandey, 2013).**

-Antioxidant and Anti-inflammatory

Polyphenols can neutralize free radicals and reduce inflammation, potentially protecting against oxidative stress and chronic diseases **(Ayse and *al.*, 2019).**

-Disease Prevention

Studies suggest a link between polyphenol-rich diets and reduced risk of conditions like diabetes, heart disease, obesity, and certain type of cancer **(BabyKC, 2023).**

-Neurodegenerative protection

Some research indicates polyphenols, like those in tannins, may help protect against neurodegenerative diseases **(Luciana and *al.*, 2023).**

-Cancer Treatment

Polyphenols, including those in curcumin, may be used as adjuvants in cancer treatment or have preventative effects **(Daria and *al.*, 2024).**

II.5.2. Food Industry

-Food Preservation

Polyphenols can be used as natural food preservatives due to their antimicrobial and antioxidant properties **(Hammad and *al.*, 2022).**

-Improving Food Properties

They can be used to enhance the color, aroma, and flavor of food products (**Prangya and Sumeeti, 2022).**

II.5.3. Industrial Applications

-Pharmaceuticals

Polyphenols are being explored for their potential in pharmaceuticals and drug development **(Wang and *al*., 2022).**

Materials Science

They can be used in the creation of hydrogels and nanocomplexes **(Fàbio and *al*., 2021).**

Chapter III

Avian pathogenic *Escherichia Coli* (APEC).

Chapter III

III.1. Generality

The bacteria pathogenic *Escherichia coli* was discovered by German pediatrician Theodor Escherich (1857–1911), who isolated it from babies' feces in 1885*. E. coli* is a gram-negative, non-sporulating, rod-shaped, facultative anaerobic, and coliform bacterium pertaining to the genus *Escherichia* that commonly inhabits the environment, foods, and warm-blooded animals' lower gut **(Shulman and *al.*, 2007).** The well-characterized Gram-negative bacterium *Escherichia coli* is present in upper respiratory and digestive tracts of poultry and mammals **(pokharol and *al.,* 2023).** Conversely, a possible risk factor for colibacillosis in poultry is the existence of pathogenic *E. coli* in the environment, digestive tract, or respiratory system **(joseph and *al.*, 2023).** Avian pathogenic *E. coli* (APEC) causes colibacillosis in poultry; this type of bacteria is an extraintestinal pathogen *E.coli* (ExPEC) **(newman and *al.,* 2021).** These conditions indicate colibacillosis including (cellulitis, omphalitis, pericarditis, perihepatitis, and salpingitis). The environment and the host's stress levels determine disease transmission; organs can still be infected, and colibacillosis can result from less pathogenic strains with fewer virulence genes **(meguenni and *al.,* 2019).**

III.2. Scientific classification

Domain: Bacteria
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma proteobacteria
Order: Enterobacterales
Family: Enterobacteriaceae
Genus: Escherichia
Species: *Escherichia coli (E. coli)* **(Escherich,1886).**

III.3. Antigenic Structure

APEC is classified into 150–200 serotypes or serogroups based on 3 antigens, somatic (O) or cell wall antigen, capsular (K) antigen, and flagellar (H) antigen. Seventy five types of the H or flagellar antigen and 173 types of O or somatic antigens 103 types of the K or capsular antigens have been recognized **(Lui and *al.,*2019) (Fig10).**

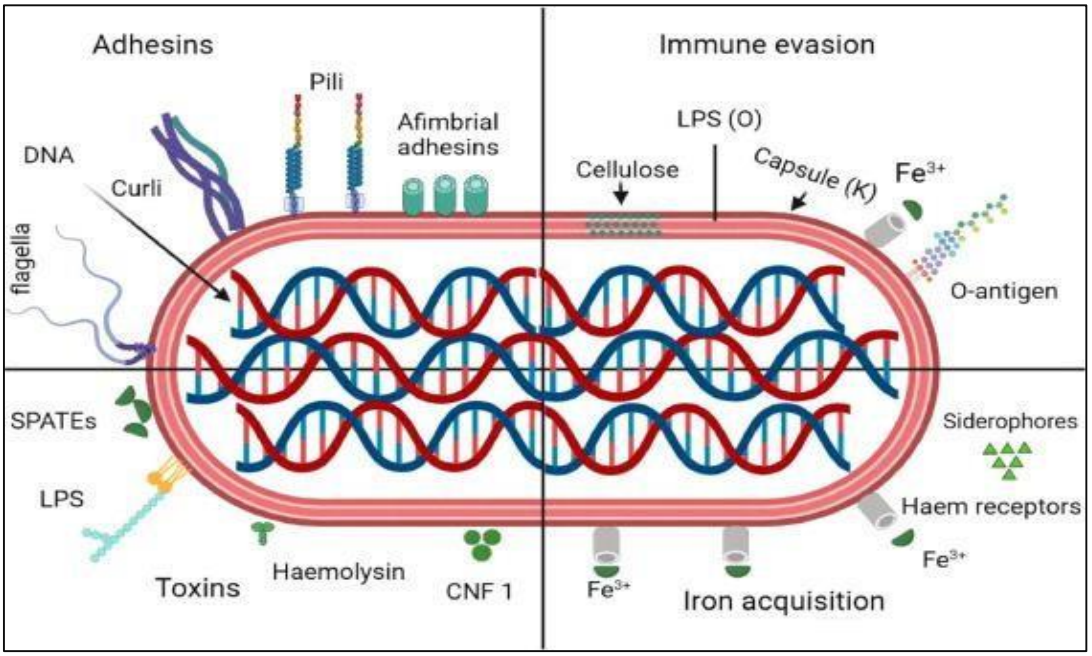


Figure 10: Structure of Avian pathogenic *E. coli* **(Miajlovic and Smith, 2014).**

III.4. Cultural requirements

APEC cells may grow on a solid or in a liquid growth medium under laboratory conditions. It may be grown in a basic minimum of media, which includes glucose as a carbon and energy source, ammonium salts as a nitrogen source, other salts, and trace elements. As *E. coli* have simple nutritional requirements it can be easily cultured on a common medium, such as Nutrient agar, Mac Conkey agar, and EMB agar **(Sesonov and *al.*, 2007).** E.coli can grow at temperatures ranging from 10°C to 40°C, although the optimum temperature for most strains is 37°C (98.6°F), however, some laboratory strains can proliferate at temperatures as high as 49°C (120.2°F) **(Catipoglu and *al.*, 2023).** E. coli can survive at 4.5-9.5 pH but the maximum growth is observed at 7.0, i.e., neutral pH. Also , the pH requirements vary with the strains of *E. coli* **(Mathlouthi and *al.*, 2018).**

III.5. Classification of pathogenic *Escherichia coli* Strains

E. coli strains can be classified as either commensal or pathogenic. The diagram in **(Fig 11)** presents the two main categories of pathogenic *E. coli*: intestinal pathogenic *E.coli* (InPEC), which are responsible for infections of the digestive tract, and extraintestinal pathogenic *E. coli* (ExPEC), which are involved in systemic or localized infections outside the intestine. Each category is further divided into several pathotypes based on their mode of action and site of infection **(Kaper and *al.*, 2023) (Fig 11).**

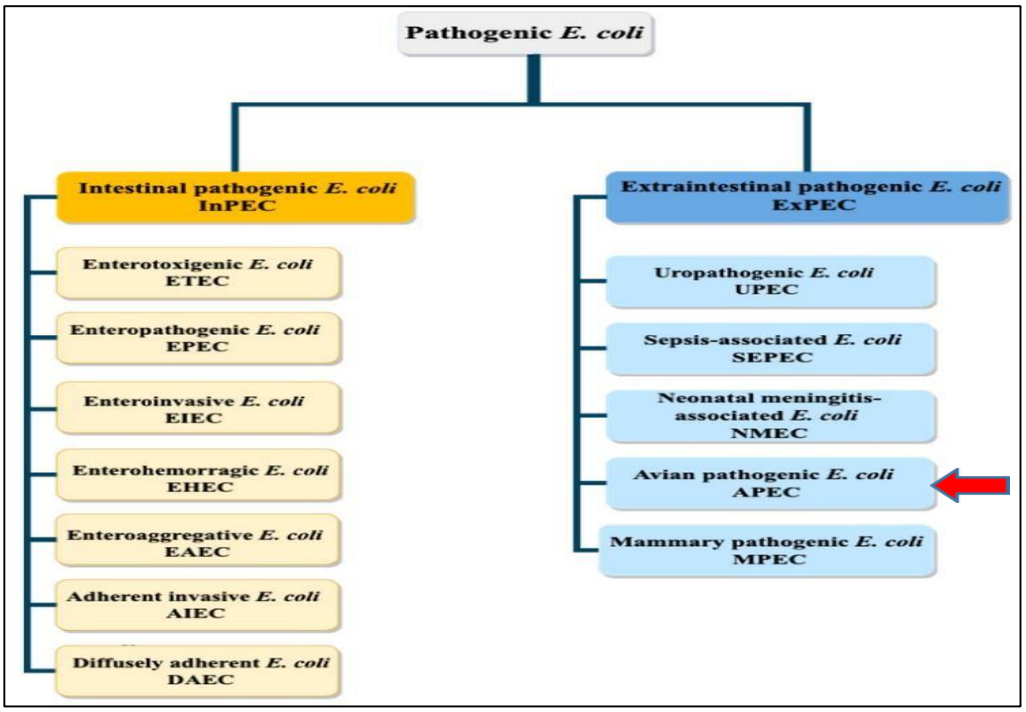


Figure 11: Pathogenic *Escherichia coli* Strains **(Khan and al., 2024).**

III.6. Characteristics

Unlike other *E.coli* pathogen groups, the characteristics of APECs cannot be identified by a single group. The following summarizes a few phenotypic and genotypic traits connected to this class of diseases. Serotyping and biotyping are frequently performed for isolates found in colibacillosis infections **(Khairullah and *al.*, 2024).**

The predominant serogroups of E. coli recovered from sick birds are O1, O2, and O78.Thus, representative serotype strains offer a focal point for deciphering the mechanisms of APEC pathogenicity and for creating and assessing potential vaccines. Given that this dominant serogroup may also be recovered from the feces of birds that appear to be in good health, the hypothesis that the digestive tract may serve as a substantial natural reservoir for APEC and that predisposing factors may be necessary to produce the disease is supported **(Ievy and *al.*, 2020).** Many investigations have revealed similarities between commensal *E. coli* and APEC, including serogroup, suggesting that APEC developed from commensal *E. coli* after gaining pathogenic qualities **(Kazibwe and *al.*, 2020).** The various features observed include the outer membrane protein profile, pathogenicity profile, and multilocus enzyme electrophoresis within a serotype. To define APEC isolates, tests for motility, hemolysis, lactose fermentation, complement resistance, serotyping, aerobactin and colicin V synthesis,

and embryo lethality are occasionally performed, although phenotypes can vary **(Wang and *al*., 2022).**

III.7. Epidemiology

APEC epidemiology in poultry is shaped by the interaction of host vulnerability, environmental factors, and bacterial genetic diversity. Factors such as young age and stress increase susceptibility, while poor hygiene and overcrowding promote bacterial transmission **(Abdelhamid and *al*., 2024) .**The pathogen's adaptability is driven by multiple virulence factors and horizontal gene transfer mechanisms, including plasmids and bacteriophages, facilitating the spread of virulence and antibiotic resistance genes **(Feng and *al*., 2023).** Continuous monitoring and strong biosecurity measures are essential to control infection and limit outbreaks in poultry production **(Wibisono and *al*., 2022).**

III.8. Transmission

APEC is a major cause of colibacillosis in poultry, affecting the poultry industry worldwide. The most frequently isolated serotypes globally are O1, O2, and O78, commonly found in Europe, North America, Asia, and Latin America. In Africa, in addition to these serotypes, strains such as O111 and O45 are also observed, often associated with high levels of antibiotic resistance.This distribution highlights significant regional diversity, influenced by Farming practices, Antibiotic usage, and the level of biosecurity **(Nawaz and *al*., 2024) (Fig12).**

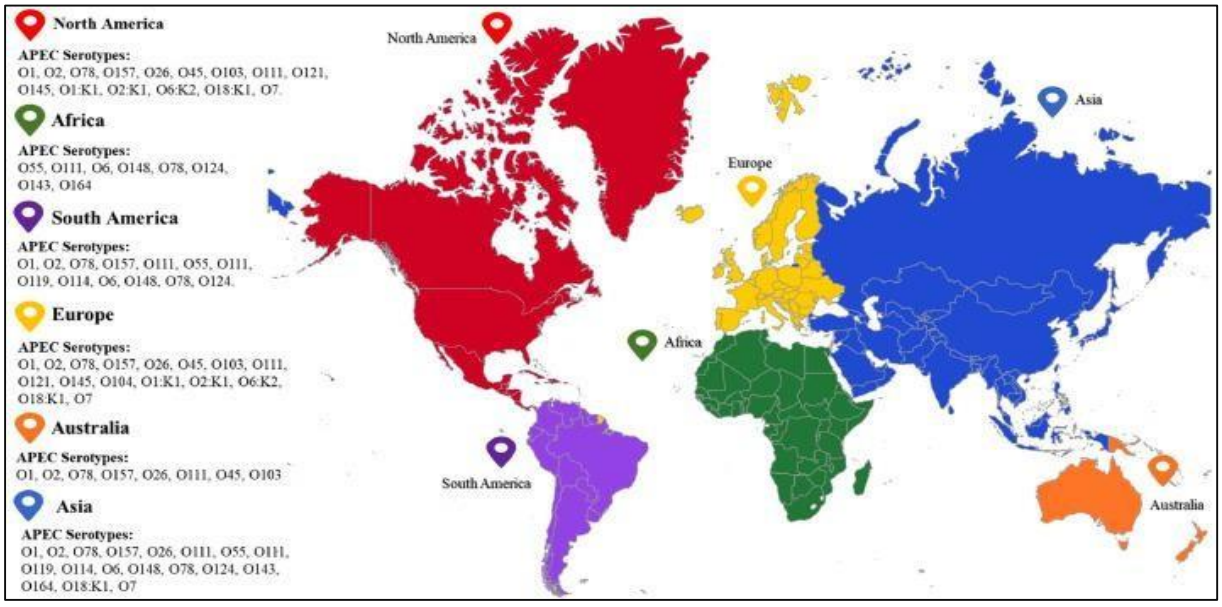


Figure 12: World map illustrating the continents with APEC serotypes **(Nawaz and *al.*, 2024)**

III.8.1. Housing conditions, ventilation, and stress

The impact of unfavorable environmental conditions has also been documented despite direct contact being demonstrated to be a significant element in transmission in APEC **(Zhou and *al*., 2024).** The respiratory systems of birds are harmed by inadequate ventilation, high dust concentrations, or other chemical vapors in chicken houses **(Wang and *al.,* 2023).** Scratches or wounds in the injured respiratory tract can allow APEC to enter, leading to the development of airsacculitis, polyserositis, and potential septicemia. Excessive ammonia concentrations can weaken the cilia lining the epithelium, impairing the bird's ability to filter dust and dangerous pathogens from its respiratory system **(Wang and *al.*, 2022).** High ammonia levels are typical because ventilation is decreased in colder climates to reduce heating expenses. Reduced ventilation leads to moist air inside the poultry house, which raises the water content in the droppings and provides a perfect environment for bacteria to release large amounts of ammonia and break down uric acid **(Van Limbergen and *al*., 2020).** This can damage the bird's respiratory system and increase the possibility of APEC transmission.

III.8.2. Contaminated water, feed, and eggs

Water could play a significant role in APEC's dissemination to poultry. Pathogenic E. coli serotypes can be introduced into chicken flocks through contaminated well water, leading to APEC transmission **(Joseph and *al.*, 2023).** The spread of APEC can be assisted by urban chicken farms that use recycled wastewater **(Walker and *al.*, 2020).** *E. coli* bacteria isolated from final effluent released from two wastewater treatment plants in the Eastern Cape Province, South Africa, were multidrug-resistant. If water is used for chicken rearing, the final effluent discharge may represent an equally significant risk **(Koutsianos and *al*., 2021).** There is a possibility that contamination of feed and feed ingredients could result in the emergence of novel disease strains. In addition, there is evidence that the type of food fed to hens affects their intestinal microbiota **(Artdita and *al*., 2021).** It has been demonstrated that some food ingredients encourage the growth of specific gastrointestinal bacteria while inhibiting the growth of other bacteria.

III.8.3. Underlying chicken disease

Colibacillosis frequently coexists with other illnesses, such as Mycoplasma and respiratory virus infections, making diagnosis and treatment challenging for farmers

(Pokharol and *al.,* 2023). Conversely, healthy hens have a strong immune system and are naturally resistant to E. coli, which is present naturally**.** Livestock can be more vulnerable to APEC infections if they have immunity abnormalities brought on by acute infections, specifically infectious bursal disease, adenovirus Newcastle disease, reovirus, Marek's disease, and infectious bronchitis. Furthermore, the role of underlying illnesses and serum antibodies against viruses such as Newcastle disease and infectious bronchitis virus in the spread of APEC are currently being studied **(Hess and *al.,* 2022)**.

III.8.4. Proximity to other animals, poultry farming, and poultry density

The risk factor for the transmission of poultry diseases is the distance between poultry farms. Reducing the number of chicken farms in a region and the number of chickens on each farm is one of the most effective ways to prevent colibacillosis **(El-Saadony and *al.*, 2022).** If biosecurity regulations are not strictly implemented, backyard flocks of chickens that usually coexist with wild birds should be separated from commercial chickens**.** It was reported that between 2002 and 2003, private flocks in the United States experienced an outbreak of exotic Newcastle disease, which later spread to commercial chicken flocks. Backyard flocks can spread zoonoses and other highly contagious infectious diseases to commercial poultry and are frequently exposed to avian influenza. The possibility of APEC transmission in poultry is thought to be increased by interactions between maintained birds and other chicken species, particularly backyard flocks. However, APEC transmission differs from that of avian influenza and Newcastle disease viruses **(Mace and Knight, 2024)**.

III.8.5. Vertical transmission

E. coli oviduct infection is a common cause of mortality in laying hens and broilers and egg production. Vertical transmission has previously been demonstrated as a means by which bacteria with AMR genes can spread **(Joseph and *al.,* 2023)**. Vertical transmission has also been demonstrated in other bacterial species, such as Salmonella enterica and Enterococcus faecalis. For the 1st time, Giovanardi documented vertical transmission of APECs from parent to offspring; previously, E. coli studies only examined outbreaks. Peterson and Bortolaia later reported the transmission of *E. coli* resistant to fluoroquinolones, nalidixic acid, and ampicillin from parent to broiler. Vertical transmission of APEC has been documented by isolating *E.*

coli clones from salpingitis-peritonitis lesions in broiler broodstock. The transmission of *E. coli* to day-old chicks is linked to increased risk **(Christensen and *al.*, 2021) (Fig13).**

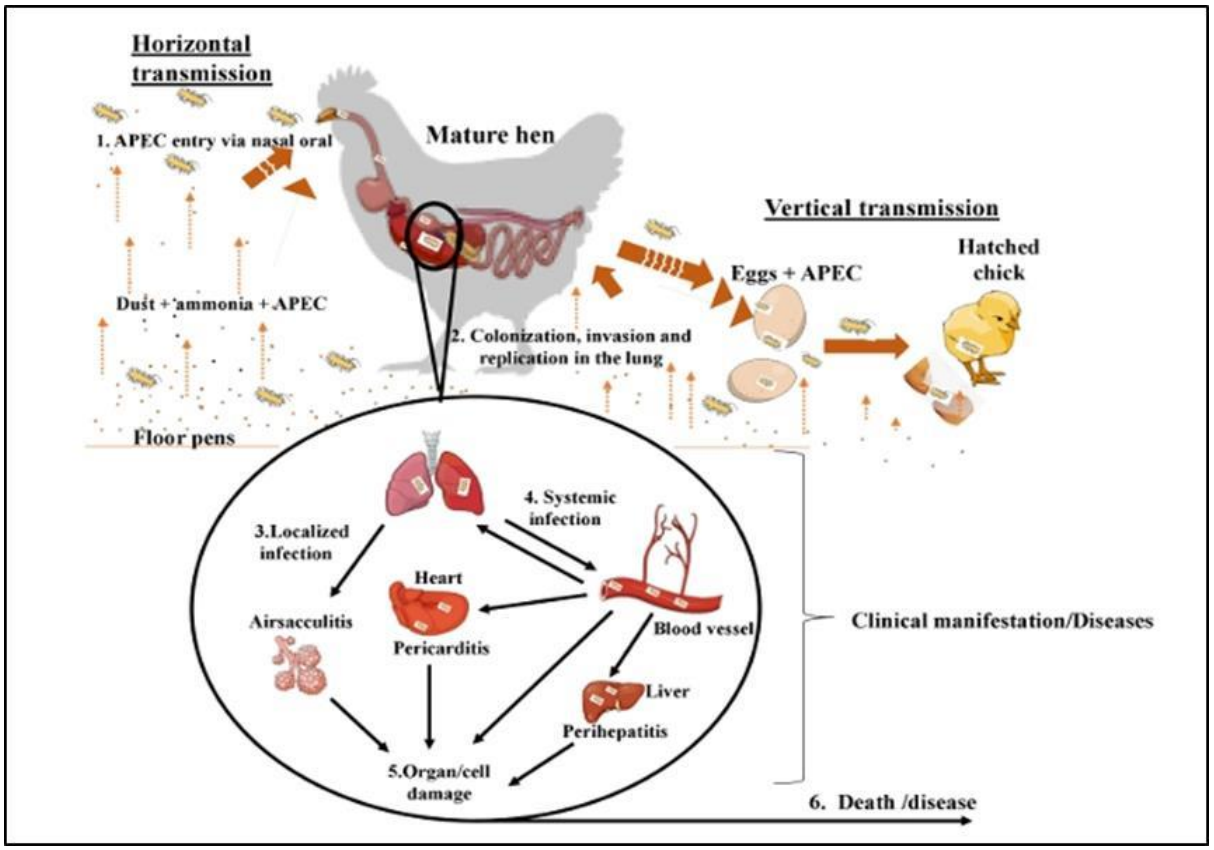


Figure 13: A schematic representation of avian pathogenic *Escherichia coli* infection in chickens after entering through oral, nasal, or cloacal routes **(Nawaz and *al.*, 2024).**

III.9. Diagnosis

III.9.1. Pathogenicity of one-day-old chicks

The most reliable method for identifying virulence in an E. coli strain is to perform a pathogenicity test on one day-old chicks **(Widodo and *al.*, 2022).** The virulence of this strain was assessed using the APEC subcutaneous inoculation method on one-day-old embryos or chicks, based on a 50% fatal dose **(Nicolas and *al.,* 2023).** This technique can be used to isolate APEC from infected chicks. In addition, the pathogenicity of APEC strains can be evaluated in 2-4-week-old chicks. This technique allows the illness to spread while it is in the field. This approach involves inoculating the sample into the nasopharynx or trachea following an initial challenge with a different agent. This is because *E. coli* is typically caused by secondary infections caused by mycoplasma, infectious bronchitis virus, Newcastle disease, or environmental factors **(Bhuiyan and *al.*, 2023).**The typical clinical manifestations of

colibacillosis include internal organ contamination, hemorrhage, pericarditis, perihepatitis, fibrin in the air sacs, and weight loss **(Apostolakos and *al.,* 2021).** This model verified that the injected strain was pathogenic.

III.9.2. Isolation and biochemistry

Most microorganisms require culture media to grow in vitro. Some of these mediums are used to support the development of microorganisms and distinguish one sample from another according to its biochemical properties. Furthermore, because they are used to distinguish closely related organisms, selective culture medium permits the growth of certain diseases and prevents the growth of other diseases **(Apostolakos and *al*., 2021).**

III.9.3. Serotype

Within the serogroup, APEC strains are categorized as serotype O: K:H **(Koutsianos and *al*., 2022).** Somatic antigens belong to serogroup O, capsular antigen to K1, flagella antigens to serogroup H, and antigens type 1 (F1A), P (F11), and curli fimbriae related to fimbriae **(Wibisono and *al*., 2022).** APEC strains include 177 “O” antigens, 100 “K” antigens, and 56 “H” flagellar antigens **(Sora and *al*., 2021).** In contrast, a different study found that APEC has 167 “O,” 74 “K,” 53 “H,” and 17 fimbriae antigens.

III.9.4. Enzyme-linked immunosorbent assay (ELISA)

ELISA is widely used in veterinary medicine as a diagnostic tool and quality control measure to detect specific pathogens like E. coli in food products **(Bai and *al*., 2023).** For research, this technique is also employed as an official veterinary diagnostic test to identify particular antigens or antibodies in production animals (such as those associated with Avian Influenza, Newcastle disease, Aujeszky’s disease, and classical swine fever) **(Mason and *al*., 2024).**

III.9.5. Molecular detection

The expansion of molecular biology tools to evaluate the genetic variability of many strains of bacteria, such as *E. coli*, occurred due to the discovery that prokaryotic genomes contain repetitive sequences: Repetitive extragenic palindrome, palindromic unit sequences, and enterobacterial repetitive intergenic consensus sequences **(Sukrama and *al.,* 2023).** The PCR reaction produces an amplified band pattern

unique to each strain; subsequent use of particular primers that are homologous to this region **(Koutsianos and *al*., 2022).**

III.10. Control Strategies

The control of APEC infections in poultry relies on antibiotic medication and vaccination, other than managing the environmental stressors, applying the biosecurity measures, and vaccinating the chickens against the viral and immunosuppressive diseases**.** Probiotics, bacteriophages, and different new alternatives (innate immune stimulants, virulence and growth inhibitors, and antimicrobial peptides) have been also evaluated with a goal to develop effective preventative and therapeutic treatments to control colibacillosis in chickens. Potential checkpoints for controlling APEC infection in chickens **(Christensen and *al*., 2020)**.

III.10.1. Management and Biosecurity Measures

The management of environmental stressors such as ammonia and dust in poultry houses by maintaining good litter and air quality are some of the key factors in preventing APEC infections in poultry houses **(Ivulic and *al.,* 2023)**. Proper ventilation as well as maintaining optimum temperature, humidity, and bird density help mitigate environmental stress in chickens. Furthermore, the elimination of pre-disposing factors by vaccinating chickens against MG, IBV, NDV, and IBD reduces the incidence of APEC infections. Good nutrition and birds with enhanced immune systems are also likely contributors to reducing the incidence of colibacillosis. Moreover, the vertical transmission of APEC can be prevented at the breeding level or at the top of the production pyramid by different intervention measures such as developing breeds with increased resistance to APEC infections, cleaning and disinfection of hatching eggs, and minimizing the use of floor eggs **(Wang and *al.*, 2024).** The horizontal transmission of APEC can be limited by using all-in-all-out production systems, systematic culling of weak chicks at first week, and implementing effective sanitation programs. The proper and efficient biosecurity measures together with feed and water (chlorination) decontamination and disinfection of poultry houses, feed mills, farm equipment, and premises are necessary to prevent APEC entry into farms. The biosafety measures such as preventing access of vectors such as houseflies, wild birds, and rodents are also necessary to keep APEC out of poultry facilities **(Christensen and *al.*, 2020).**

III.10.2. Antibiotics

Antibiotics are commonly used to control APEC infections in poultry. Many antibiotics belonging to different classes, such as tetracyclines (tetracycline, oxytetracycline, chlortetracycline), sulfonamides (sulfadimethoxine, trimethoprim, sulfadiazine, sulfamethazine, sulfaquinoxaline, ormethoprim), aminoglycosides (apramycin, gentamicin, neomycin, spectinomycin), penicillins (amoxicillin, ampicillin), cephalosporins (ceftiofur), quinolones (danofloxacin, sarafloxacin, enrofloxacin), polymyxins (colistin), chloramphenicols (florfenicol), macrolides (erythromycin), and lincosamides (lincomycin) have been used in poultry industry worldwide for the control of APEC infections **(Christensen and *al*., 2020).**

III.10.3. Vaccines

Various vaccine candidates, mostly live-attenuated and recombinant vaccines, have been investigated to protect chickens against APEC infections. In the past, inactivated vaccines were tested; however, recent studies have focused mostly evaluating live-attenuated and recombinant vaccines in chickens **(Dai and *al.,* 2023).** The varying degrees of protection, ranging from none to partial to complete, have been achieved using these vaccines. Among the tested vaccines, multiple vaccines such as outer membrane vesicles (OMVs), bacterial ghost vaccines, recombinant iss, recombinant antigen (rAg) vaccine containing ExPEC antigens, Salmonella-delivered vaccines containing APEC antigens, ΔaroA, and ΔtonB/Δfur were able to reduce the mortality, lesions, and bacterial burden as well as stimulate the antibody (immunoglobulins; IgG and IgA) responses in chickens **(Christensen and *al*., 2020).**

III.10.4. Probiotics

Different probiotics have been tested for their efficacy to prevent APEC infections in chickens. The efficacy of Lactobacillus plantarum B1 was evaluated against E. coli (K88) infection by supplementing in the broilers feed (2 × 109 CFU/kg). Broilers fed with L. plantarum B1 showed significanly decreased cecal E. coli counts and increased growth performance, villus height to crypt depth ratio, ileal mucosal sIgA concentration, and cecal lactic acid bacteria counts. Similarly, the efficacy of L. plantarum 15-1 and fructooligosaccharides (FOS) combination was evaluated against APEC (O78) infection by supplementing in the broilers feed (1 × 108 CFU/kg) **(Ding and *al.*, 2019)**. The decrease in mortality and serum diamine oxidase and increase in IgA and IgG

concentrations was observed in broilers fed with probiotic and FOS mix. The effects of Enterococcus faecalis-1 was assessed in broiler chickens challenged with APEC (O78) by inoculating orally in drinking water for 3 days (1 × 108 CFU) from days 1 to 3 of growth [210]. E. faecalis-1 supplementation significantly improved the growth performance and immune response, reduced the mortality, and decreased the visceral organs invasion by APEC O78. Likewise, the efficacy of multi-strain commercial probiotic mix (Bacillus subtilis, Clostridium butyricum, and L. plantarum) was tested against APEC 078 infection by supplementing in the broilers feed. There was significant decrease in mortality (13.6% to 0%) and APEC counts in liver and spleen and increase in growth performance and lactobacilli population in broilers fed with the probiotic mix **(Shehata and *al.*, 2019) (Zhao and *al.*, 2018)**. Another commercial probiotic mix (B. subtilis, L. acidophilus, Pediococcus acidilactici, Pediococcus pentosaceus, and Saccharomyces pastorianus) was also tested in combination with recombinant attenuated Salmonella vaccine (RASV) for protection against APEC (O78:K80) and Salmonella infection by supplementing in feed in layer chickens **(Christensen and *al.*, 2020).**

III.10.5. Bacteriophages

To date, multiple studies have been conducted to evaluate the preventative and therapeutic efficacy of phages against APEC infections in chickens **(Siddiqui and *al.*, 2023)**. The efficacy of phage mixture (SPR02 and DAF6) was evaluated in APEC (O2) challenged chickens by spray and intramuscular administrations. The phage treatment three days prior to APEC challenge significantly reduced (40% to 3%) the mortality of chickens. Similarly, phage treatment at 24 h and
48 h post-challenge also reduced the mortality rate (55% to below 20%) **(Wang and *al.*, 2021)**. The efficacy of phage cocktail (phi F78E Myoviridiae, phi F258E Siphoviridae, and phi F61E Myoviridae) was tested in experimentally (O78) and naturally infected flocks refractive to antibiotic treatment by oral or spray administration. The treatment with phage cocktail reduced mortality by 25% in experimentally infected chickens and decreased the flocks' mortality level to below 0.5% in flocks infected naturally with APEC. Similarly, the efficacy of another phage cocktail (TM1, TM2, TM3, and TM4) was evaluated in APEC challenged (O78:K80 and O2:K1) chickens by administering through intramuscular injection. The phage cocktail treatment reduced the mortality (46.6% to 13.6%), APEC load in lung, and APEC

lesions in lung, liver, and heart, and increased the body weight of chickens **(Christensen and *al.*, 2020).**

III.10.6. New Alternative Approaches

Apart from antibiotics, vaccines, probiotics, and bacteriophages, different novel approaches, including but not limited to innate immune stimulants, virulence and growth inhibitors, and antimicrobial peptides have been studied for their protective effects against APEC infections in chickens **(Helmy and *al.*, 2023).**

Experimental part

Materials And Methods

IV.1. Plant material

The species *Eriobotrya japonica* Lindl was collected in February 2025, in Ain Nouissy, Mostaganem **(Fig 14).**

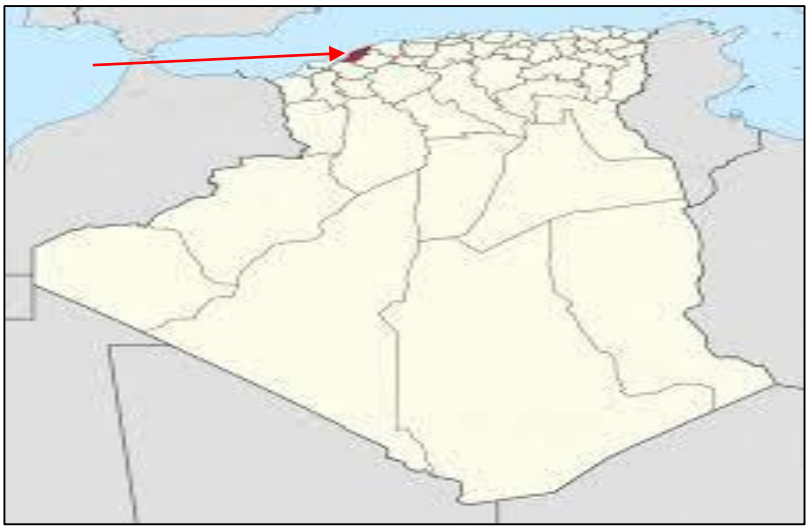


Figure 14: Geographical location of the plant collection site. https://commons.wikimedia.org/wiki/File:Mostaganem_in_Algeria.svg Consulted on june 08 2025.

Plant was identified by professor Sekkal.F, biothechnology departement, Abdelhamid Ibn badis University, Mostaganem.

The aerial part (leaves, stems) and roots were used during our work. These organs were collected, air dried at room temperature (25°C) during 48h. They were then ground into very fine particles **(Fig15).**

Figure 15: Plant material (**A** Leaves, **B** Stems and **C** Roots).

IV.2. Extraction

For each plant part leaves, stems, and roots 20 g of dried plant powder were accurately weighed and placed into a cellulose thimble. The thimble was then inserted into a Soxhlet extractor, mounted on a 250 mL round-bottom flask

containing a hydroalcoholic solution of ethanol 96° and distilled water in a 70:30 (v/v) ratio as the solvent. The flask was heated at boiling temperature , and a condenser was placed at the top of the extractor to condense the solvent vapors, allowing continuous solvent circulation **(Azmir and *al.,* 2013)**. The extraction was carried out separately for each plant part, with 8 Soxhlet cycles per extraction.

The hydroalcoholic extracts were then passed through a rotary evaporator 40°C to remove the ethanol. The resulting concentrates were poured into Petri dishes and dried in an oven at 45 °C to eliminate any residual water. After drying, the dry extracts were stored at 4 °C until further use.

IV.3. Determination of the yield

Yield refers to the amount of product obtained from a given quantity of raw material during an extraction or chemical process. It is usually expressed as a percentage.

The empty Petri dishes were weighed (W_0) , then weighed again after being filled with the dry extracts (W_1).These measurements were used to calculate the extraction yield.

Mass of dry extract = $W_0 - W_1$

Where:

W_0: weight of the Petri dish (before evaporation)

W_1: weight of the Petri dish (after evaporation)

The extraction yield (Y) can be calculated using the following equation:

Y% = (Mass of dry extract / Mass of plant material) × 100 **(Mokgadi, 2008).**

IV.4. phenolic compounds content

IV.4.1. Principle

The principle of phenolic compounds content is based on the use of the Folin Ciocalteu reagent, which is a mixture of phosphotungstic acid ($H_3W_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). Upon oxidation of phenolic compounds, these acids are reduced to a blue-colored complex consisting of tungsten and molybdenum oxides, in the presence of an alkaline solution. The intensity of the resulting blue coloration, which is proportional to the concentration of phenolic compounds in the sample, shows a maximum absorbance at 760 nm **(Slinkard and Singleton, 1977).**

IV.4.2. Protocol

The initial solution was prepared at 2 mg/mL for each extract. 400 µL of 0.5 N Folin Ciocalteu reagent were added, and then 400 µL of sodium carbonate solution (NaOH) at 10% .The mixture was incubated at room temperature in the dark during 90 minutes, to allow the development of the characteristic blue coloration of the reaction.The absorbance of each sample was measured at 760 nm using a spectrophotometer. The total phenolic content was determined by interpolation on a calibration curve prepared from gallic acid standard solutions (mg/ml), and the results were expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM) **(Slinkard and Singleton, 1977).**

IV.5. FTIR-ATR Spectroscopy (Fourier Transform Infrared – Attenuated total reflectance)

FTIR-ATR (Jasco FTIR-4700) provides information related to the presence or absence of specific functional groups, as well as the chemical structure of polymer materials. Shifts in the frequency of absorption bands and changes in relative band intensities indicate changes in the chemical structure or changes in the environment around the sample.

Thus, FTIR-ATR spectroscopy can be used to determine the resultant surface chemistry especially following induced chemical or physical modifications. **(Smith, 2011) .**The "ATR" part stands for Attenuated Total Reflectance, a sampling technique that allows for direct analysis of solid or liquid samples without the need for extensive preparation **(Grzelec, 2024) (Fig16).**

Figure 16: FTIR-ATR spectroscopy (Jasco FTIR-4700).

IV.5.1. Data Acquisition and analysis

The prepared sample is placed in the FTIR spectrometer with proper contact against the ATR crystal, ensuring correct positioning. Appropriate acquisition parameters are selected, such as spectral resolution, wavelength range, number of scans, etc. The measurement is then started by recording the infrared absorption spectra of the sample **(Zhao and *al*., 2024).**

Once the data is collected, a Fourier transform is applied to convert the raw interferogram into an FTIR spectrum. The FTIR-ATR spectrum is analyzed by identifying characteristic absorption bands corresponding to molecular vibrations present in *Eriobotrya japonica* Lindl. The resulting spectra are compared with spectral libraries or known reference spectra to identify the chemical components present in *Eriobotrya japonica* lindl **(Derksen and *al*., 2023).**

IV.6. Bacteriological exam

The antibacterial activity of the extracts was evaluated on Avian Pathogenic *E. coli*. The bacterial strains were obtained from the Microbiology Laboratory of Avian Pathologies Institute of Veterinary Sciences (Tiaret), These strains were isolated from broiler chickens showing clinical signs of infection, collected from three different poultry farms.

The isolats bacteria used for our study are mantioned in table 1.

Table 1: List of *E. coli* Strains Used in the Study.

Strain	Strain Type
E. coli ATCC 25922	Reference strain
strain 1 (21-24days)	Avian Pathogenic E. coli
strain 2 (4days)	Avian Pathogenic E. coli
strain 3 (21-24days)	Avian Pathogenic E. coli
strain 4 (4days)	Avian Pathogenic E. coli
strain 5 (21-24days)	Avian Pathogenic E. coli
strain 6 (4days)	Avian Pathogenic E. coli
strain 7 (21-24days)	Avian Pathogenic E. coli

IV.6.1. Culture media preparation

Three culture medium were prepared (according to the manufacter's recommendation): two medium for culture (nutrient agar, MACConkey) and Mueler-Hinton medium for performing the antibiogram.

IV.6.2. Identification of bacteria

The samples were inoculated into Petri diches containing MACConkey agar.This culture medium has the advantage of growing all enterobacteria, including *Escherichia coli*. The various inoculated disches were then incubated at 37°C for 24hours.The colonies that appeared were observed macroscopically, then one colony was selected and cultured in tubes containing BHIB (Brain Heart Infusion Broth" or "BHI broth").

These tubes were then incubated at 37°C in an incubator for 24 hours. Each pure culture was subjected to a gram stain.

IV.6.3. Gram stain's principle

The principle of Gram staining relies on the ability of the bacterial cell wall to retain the crystal violet dye after decolorization with a solvent. Gram-positive bacteria retain the crystal violet stain due to their thick peptidoglycan layer, which prevents the dye from being washed away. Gram-negative bacteria lose the crystal violet stain and can be counterstained with safranin, appearing red or pink.

Gram-negative bacteria, having lost the crystal violet, can be stained with safranin, a pink or red dye.

The observation of stain bacteria under a light microscope using the oil immersion objective (Gx100).

IV.7. Disk diffusion (Antibiogram).

This method allows to determined the resistance and sensitivity of isolats bacteria and reference strains against antibiotics (Gentamicin, Tetracycline, Co- trimoxazole, Cefotaxime), a young bacterial colonies suspension are prepared.The turbidity of this suspension is adjusted to 0.5 McFarland, equivalent at optical density 0.08-1. This inoculum is spread using a swab onto Petri dishes containing Mueller-Hinton agar. Then antibiotic impregnated discs table2 were placed on the surface of the pre-inoculated media. The plates were incubated at 37°C for 24 hours to allow the antibiotics to diffuse into the medium and to evaluate their inhibitory effect on bacterial growth **(Balouirit and *al.*, 2016 ; CLSI., 2021)(Fig 17,Tab2).**

Table 2: Antibiotics Used **(Kohanski and *al.*, 2010).**

Antibiotic	Class	Target	Susceptible (S)	Intermediate (I)	Resistant (R)
Gentamicin	Aminoglycosides	Inhibits protein synthesis	≥15 mm	13–14 mm	≤12 mm
Tetracycline	Tetracyclines	Inhibits protein synthesis	≥19 mm	15–18 mm	≤14 mm
Co-trimoxazole	Sulfonamides + Trimethoprim	Inhibits folic acid synthesis	≥16 mm	11–15 mm	≤10 mm
Cefotaxime	Cephalosporins	Inhibits cell wall synthesis	≥23 mm	15–22 mm	≤14 mm

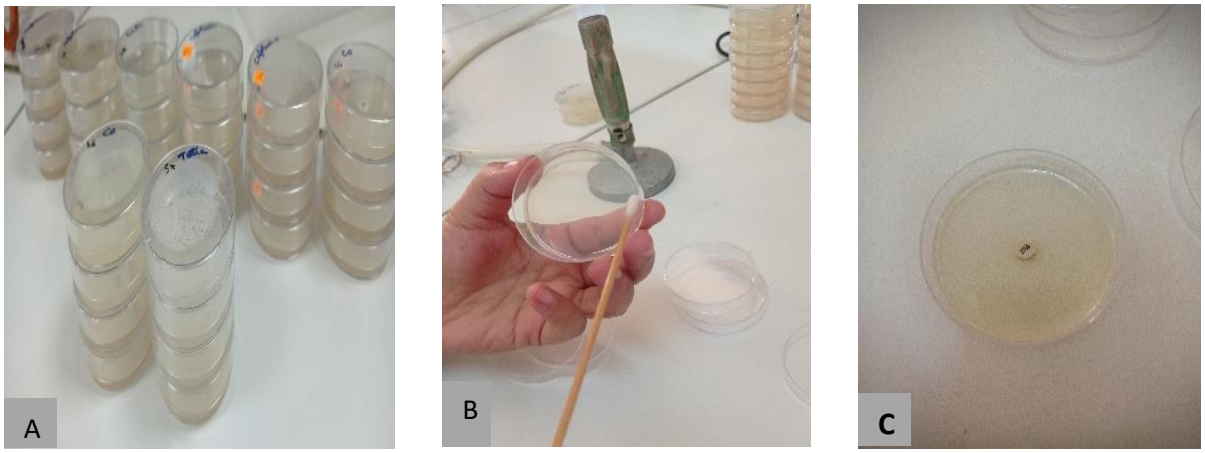


Figure 17: Antibiogram of antibiotics.**A:** Pouring of Petri dishes with Mueller-Hinton agar.**B:** Inoculation of bacterial strains.**C:** Antibiotic Disc Impregnation.

Selection of these antibiotics due to their broad spectrum of activity and proven effectiveness against *E. coli* and other Gram-negative bacteria, as reported in the National Standardization of Antibiotic Susceptibility Testing guidelines (INSP, 6th edition, 2011, p.131).

IV.8. The microdilution test

The microdilution test in 96-well microplates, based on the WHO (2002) method, was used to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC), in order to assess the antibacterial effects of study extract.

We added 100 µL of Mueller-Hinton Broth (MHB) to all the wells of the microplate. Then, we introduced 100 µL of the initial solution of the extract into the first well. After we were realised dilutions at ½ (400, 200, 100, 50, 25, 12.5 ,6.25 mg/ml). Subsequently, we added 20 µL of the standardized bacterial suspension to each well. Finally, the microplates were incubated at 37 °C for 24 hours **(Klancnik and *al.*, 2010) (Fig18).**

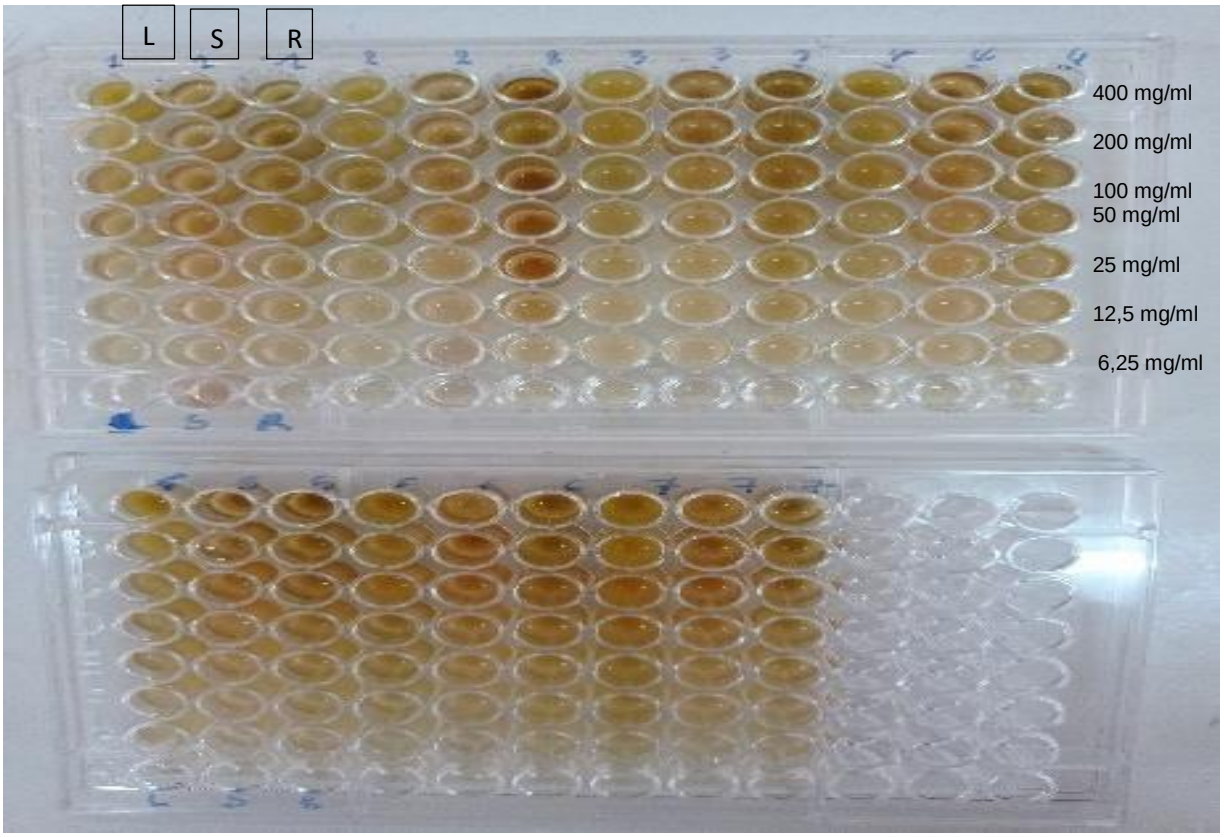


Figure 18: Microdilution test.

IV.9. Determination of MIC, MBC

The MIC of an extract against bacterial strain presents the lowest concentration that shows no visible bacterial growth to the naked eye **(Balouiri and *al.*, 2016).** The Minimum Bactericidal Concentration (MBC) was determined by subculturing the contents of all the wells, after the MIC determination, onto Mueller-Hinton agar and incubating at 37 °C for 24 hours.The lowest concentration of the extract that did not allow any bacteria to survive corresponded to the MBC.antibacterial activity extracts was determined by calculating the ratio of the MBC to the MIC. If the ratio is greater than or equal to 4, the extract is considered bacteriostatic; if it is less than 4, the extract is considered bactericidal **(Balouiri and *al*., 2016).**

Results
and
Discussion

V.1. Determination of extraction Yield

The highest yield was obtained from the leaves (17.15%), followed by the stems (11.35%) and the roots (9.65%) **(Tab3).**

Table 3: Extraction Yield of Different Parts of Plant.

Plant Part	Dry Extract Weight (g)	Initial Sample Weight (g)	Yield (%)
Leaves	3.43	20	17.15 %
Stems	2.27	20	11.35 %
Roots	1.93	20	9.65 %

V.2. Phenolic compounds content

According to the calibration, the following equation was obtained:

Y=1,6002x with R^2= 0,9949 **(Fig19)**.

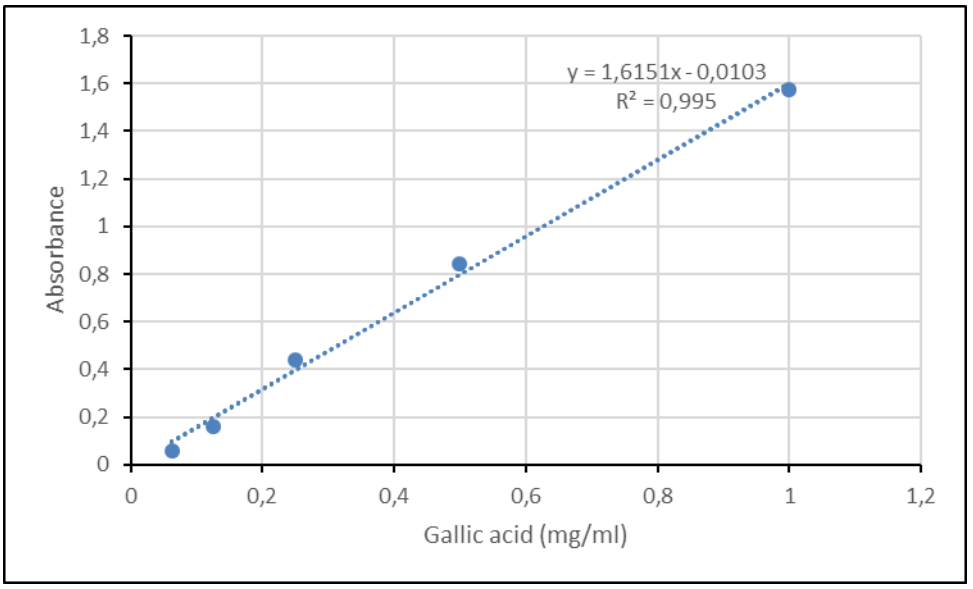


Figure 19: Calibration curve of gallic acid.

The results show a significant variation in total polyphenol content across different organs of *E. japonica*. The stem presented the highest concentration (1.9 ± 0.7 mg AGE/gDM), the leaves displayed an intermediate polyphenol content (1.5 ± 0.4 mg AGE/gDM), and the roots the lowest (1.1 ± 0.8 mg AGE/gDM) **(Fig20).**

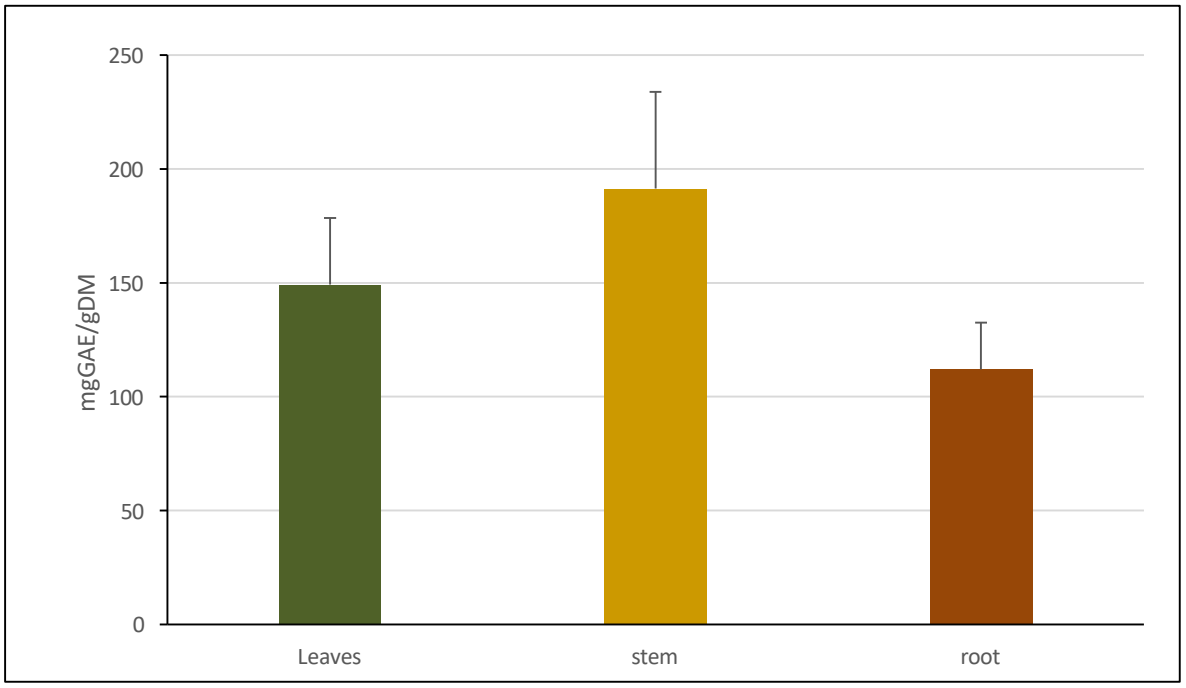


Figure 20: Total Polyphenol Content (expressed as absorbance) in Different Organs of *Eriobotrya japonica* Lindl.

V.3. Analysis (FTIR -ATR) Spectroscopy

V.3.1. Leaves

Spectroscopy (FTIR-ATR) analysis enables the detection of functional groups present in the compounds extracted from the leaf. This includes polyethers such as polyethylene oxide and poly(tetramethylene oxide), characterized by ether bonds (–C–O–C–). Alkanes (R-CH_2-R) and aromatic compounds (Ph-CH_3) are also identified, as well as hydrazines (-NH-NH_2), which are nitrogen-containing compounds. Hydroxyl groups are represented by several types of alcohols: aromatic (Ph-OH), primary (RCH_2-OH), diols (HO-R-OH), and aromatic secondary alcohols (Ph-CHR-OH). Polyureas (-NH-C=O) are also detected. Among the important secondary metabolites like Flavonoid (Quercetin 3 rhamnoside), catechol (tannin), and several alkaloids such as α-(4'-hydroxy-4-biphenylyl)-1-pyrrolidineethanol and 2- pyridinemethanol are present. Phenolic glycosides and phenols are also found **(Fig21, Tab4,5).**

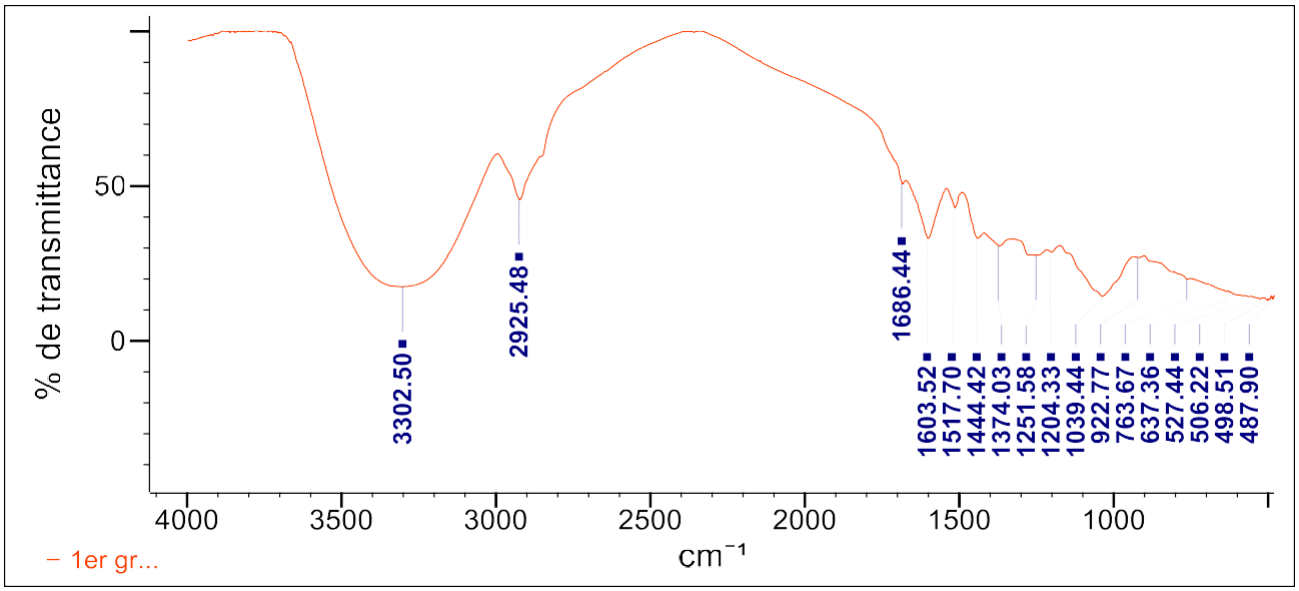


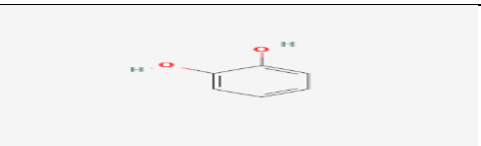
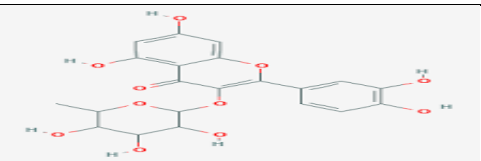
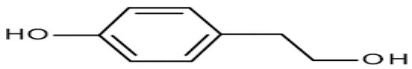
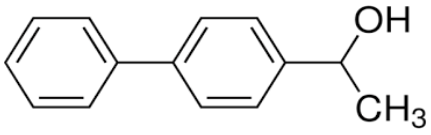
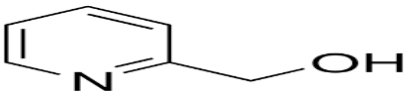
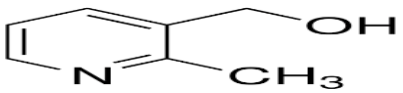
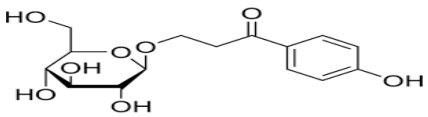
Figure 21: FTIR-ATR Graph of the leaves.

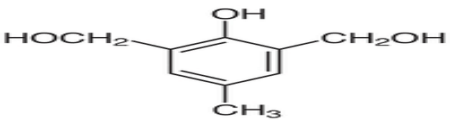
Table 4: Functional Groups of Leaves.

Functional Group	Typical Bond / Structure
Polyethers (Polyethylene oxide)	-C-O-C- (ether linkage)
Alkanes (R–CH_2–R)	-CH_2-
Alkanes (Ph–CH_3)	-CH_3
Hydrazines	-NH-NH_2
Polyethers (Tetramethylene oxide)	–C–O–C–
Alcohols (Ph–OH)	-OH on aromatic ring
Alcohols (R–CH_2–OH)	-OH on primary alcohol
Diols (HO–R–OH)	Two -OH groups

Functional Group	Typical Bond / Structure
Polyureas (Polymethylene urea)	–NH–C=O
Alcohols (Ph–CHR–OH)	Aromatic secondary alcohol

Table 5: Chemical structures found in leaves.

Compound Name	Chemical Structure	Secondary metabolite
Catechu (Catechol)		Tannins
Quercetin-3-rhamnoside		flavonoid
α-(4'-Hydroxy-4-biphenylyl)-1-pyrrolidineethanol		Polyphenolic
α-(4'-Hydroxy-4-biphenylyl)-1-pyrrolidineethanol, hydrochloride		Alkaloid
2-Pyridinemethanol		
α-Methyl-3-pyridinemethanol		
o-Hydroxyphenyl 1-thio β-D-glucopyranoside		Phenolic glucoside

Compound Name	Chemical Structure	Secondary metabolite
2,6-Bis(hydroxymethyl)phenol		Phenol

V.3.2. Stem

FTIR-ATR analysis enabled the identification of several functional groups present in the compounds extracted from the stem. Polyethers, such as polyethylene oxide, poly(tetramethylene oxide), and polyethylene glycol, are polymers characterized by ether bonds (–C–O–C–). Amine salts, represented by NH_3^+, were also identified. Alkanes are saturated hydrocarbons; in this case, both linear alkanes (R-CH_2-R) and aromatic alkanes such as (Ph-CH_3) were observed. Alcohols are compounds containing a hydroxyl group (-OH). Identified types include primary alcohols (R-CH_2-OH), aromatic secondary alcohols (Ph-CHR-OH), as well as phenols (Ph-OH) **(Fig22, Tab 6).**

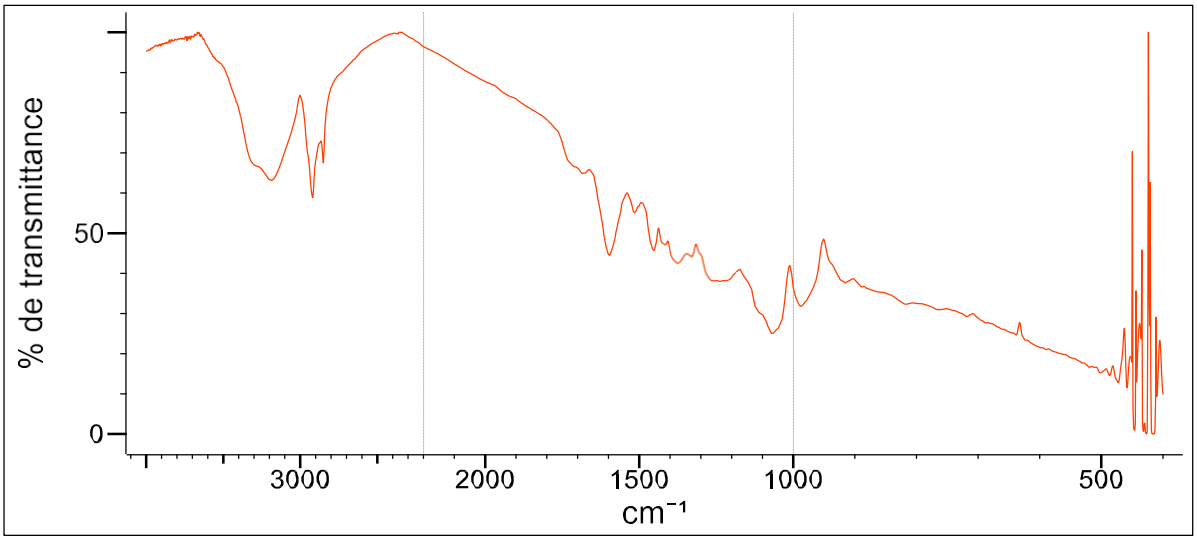


Figure 22: FTIR-ATR Graph of the stems.

Table 6: Functional Groups of stem.

Classe	Group	secondary metabolites classified
Polyethers – Polymeres	Polyethylene oxide	
	Poly (tetramethylene oxide)	
	Polyethylene glycol (crystalline)	
	Polyethylene glycol (amorphous)	
Amine salts	NH_3^+	
Alkanes	$R\text{-}CH_2\text{-}R$	Linear alkanes
	$Ph\text{-}CH_3$	Aromatic alkanes
Alcohols	$R\text{-}CH_2\text{-}OH$	Primary alcohols
	$Ph\text{-}CHR\text{-}OH$	Aromatic secondary alcohols
	$Ph\text{-}OH$	Phenols
Phenol	-OH	Phenolic compound Lignin

V.3.3. Roots

FTIR-ATR analysis enabled the detection of various characteristic functional groups present in the root extract. Among these, aromatic secondary alcohols of the type (Ph-CHR-OH) are classified as polyphenols. Carboxylic acids (RCOO), particularly those with phenolic characteristics, were also identified. Additionally, nitro compounds (O-NO_2, N-NO_2, C-NO_2), which may originate from either natural or synthetic sources, were detected. Nitrogen-containing compounds such as hydrazones (CH=N-NH_2) and azines (RCH=N-N=CHR) were present as well. The analysis also revealed the presence of amidines (N=CH-N) were identified among the detected functional groups **(Fig23, Tab 7).**

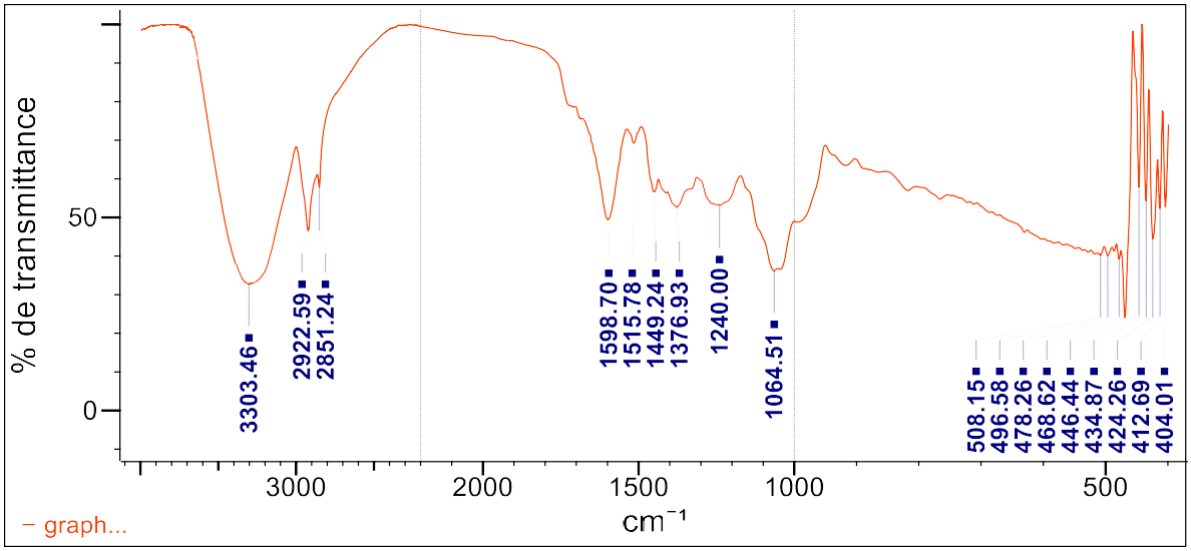


Figure 23: FTIR-ATR Graph of the roots.

Table 7: Functional Groups of roots.

Classification	Group	secondary metabolites classified
Alcohols	Ph-CHR-OH	Aromatic alcohols
Terpenoids coumpounds	-OH -COOH -O-	Saponin
Terpenoids coumpounds	-OH -COOH -O-	Triterpen
Phenolic compounds	-OH	Cumarin
Nitro Compounds	O-NO_2	
Nitro Compounds	N-NO_2	
Carboxylic Acids	RCOO	phenolic acids
Nitro Compounds	C-NO_2	
Azines	RCH=N-N=CHR	
Hydrazones	CH=N-NH_2	
Amidines	N=CH-N	

V.4. Identification of Bacteria

V.4.1. Macroscopic observation

On MacConkey agar, we observed that *Escherichia coli* formed pink to red colonies.

V.4.2. Microscopic observation

After Gram staining, we observed under the microscope that *Escherichia coli* appeared as small Gram-negative bacilli, rod-shaped, straight or slightly curved. The cells were arranged singly, in pairs, or occasionally in short chains, and were non-spore-forming **(Fig24).**

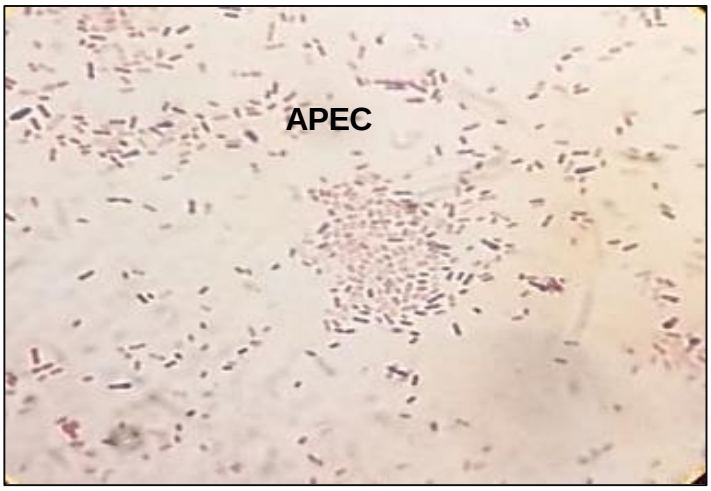


Figure 24: Microscopic observation of APEC *(*G x100, gram stain).

V.5. Disk diffusion (Antibiogram)

The results measuring the diameter of these zones According to the document entitled "Standardisation of Antibiotic Susceptibility Testing at the National Level (Human and Veterinary Medicine)," (6th Edition, WHO, 2011)**.**

the sensitivity tests show that all APEC strains studied exhibit high sensitivity to cefotaxime, with inhibition zone diameters ranging from (25 to 29 mm).

Strains 2, 4, 6 and atcc were sensitive (18-19 mm), while others (1, 3, 5 and 7) were intermediate to gentamicin (13-14 mm). all strains were resistant to tetracycline, with low inhibition zones (10-14 mm), and to co-trimoxazole (3-9 mm), In contrast, reference strain was intermediate to tetracycine with inhibition zone 16 mm also to co-trimoxazole with 11 mm. **(Tab8, Fig25).**

Table 8: Inhibition Zone Diameters (mm) of Antibiotics Against *E. coli* Strains.

Strain/Antibiotic	Cefotaxime (mm)	Gentamicin (mm)	Tetracycline (mm)	Co-trimoxazole (mm)
ATCC (Reference Strain)	29	19	16	11
Strain1 (APEC)	25	13	10	3
Strain2 (APEC)	28	19	13	8
Strain3 (APEC)	25	13	10	3
Strain4 (APEC)	28	18	13	8
Strain5 (APEC)	26	14	11	4
Strain6 (APEC)	29	19	14	9
Strain7 (PEC)	26	14	11	5

Sensitive

Intermediate

Resistant

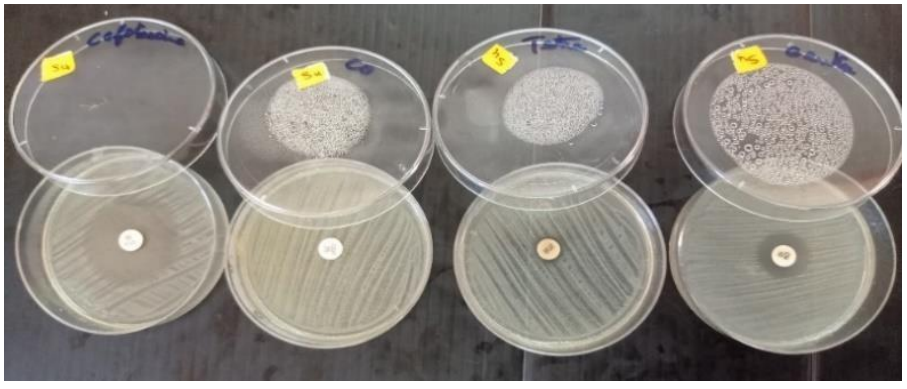


Figure 25: Inhibition zones of antibiotics in strain 4.

V.6. Antibacterial activity of *E. Japonica* by microdilution

We observed a gradual appearance of bacterial growth disturbances when moving from high to low concentrations, with the strongest effects observed in the leaf extract, followed by the stem and root extracts. Notable differences were also observed between the tested strains in terms of their sensitivity to the extracts **(Fig26).**

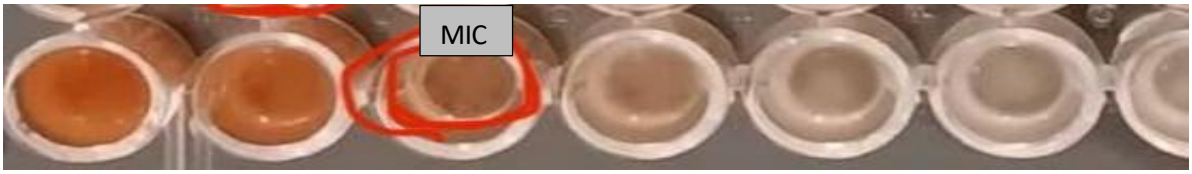
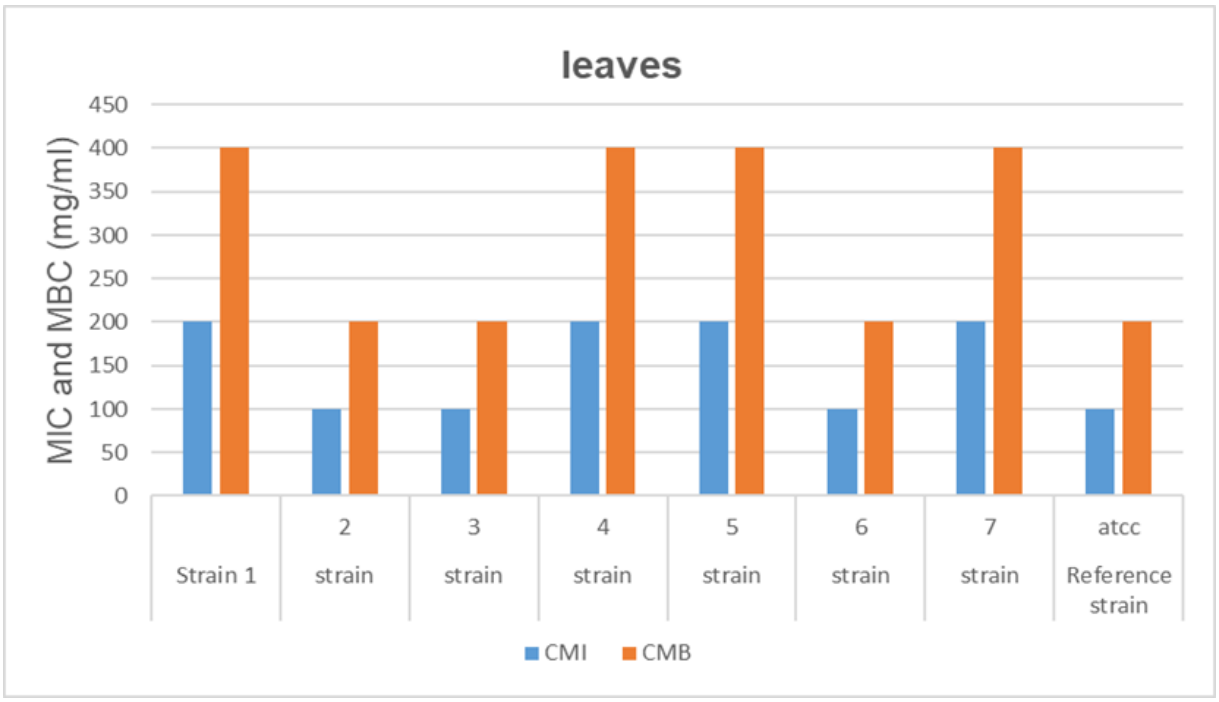


Figure 26: Minimum inhibitory concentration of leaves in reference strain.

V.6.1. MIC and MBC of leaves

MIC and MBC values tend to be lower with MIC (100 mg/mL) and MBC (200 mg/ml) in ATCC and 2, 3, 6 strains , while 1, 4, 5, and 7 exhibit higher MIC values (200 mg/ml) and MBC (400mg/ml) **(Fig27).**



Figures 27: MIC and MBC values of leaves

V.6.1.1. MBC/MIC ratio leaves.

we have a MBC/MIC ratio ≤ 4, indicating a bactericidal effect of the leaf extract on the APEC strains studied **(Tab9).**

Table 9: MBC/MIC ratio of leaves .

Strains	MIC	MBC	MBC/MIC	Effect
ATCC, 2, 3, 6	100	200	**2** < 4	bactericidal
1, 4, 5, 7	200	400	**2** < 4	bactericidal

V.6.2. MIC and MBC of stem.

MIC values were notably higher (400 mg/mL) for strains 1, 4, 5 and 7, which also showed no detectable MBC. In contrast, ATCC, 2, 3 and 6 strains showed lower MIC values (200 mg/mL), while the MBC was 400 mg/mL.

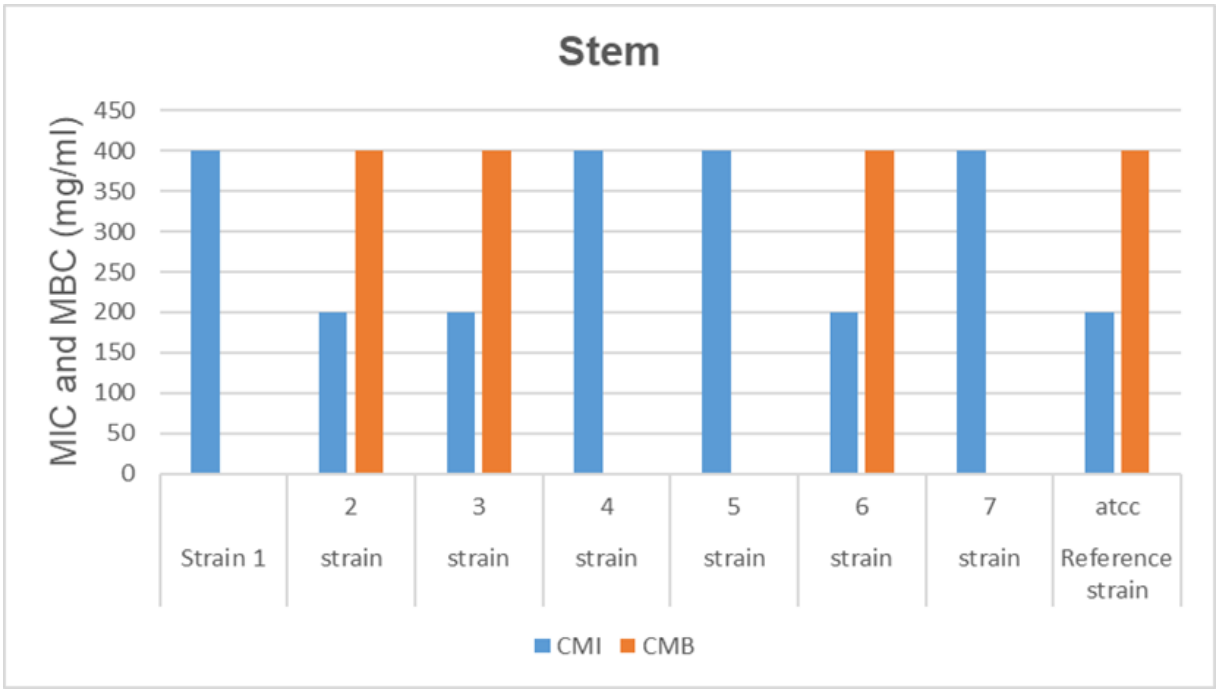


Figure 28 : MIC and MBC values of stems.

V.6.2.1. MBC/MIC ratio stems

the CMB/CMI ratio is less than 4 for ATCC, 2, 3 and 6 strains, so the stems have a bactericidal effect against these strains. For strains 1, 4, 5 and 7, only the MIC could be determined. In the absence of a measurable MBC, the effect of the extract can not

be qualified with certainty, suggesting an inhibitory activity with no apparent bactericidal effect at these concentrations (Tab10)

Table 10: MBC/MIC ratio of stems

Strains	MIC	MBC	MBC/MIC	Effect
ATCC, 2, 3, 6	200	400	**2** < 4	Bactericidal
1, 4, 5, 7	400	/	/	/

V.6.3. MIC and MBC of roots

MIC values were 400 mg/ml for ATCC 2, 3 and 6 strains, with no MBC, while strains 1, 4, 5 and 7 showed neither MIC nor MBC, so all strains have no MBC.
As no MBC values were obtained for any of the strains, the MBC/MIC ratio could not be determined **(Fig29).**

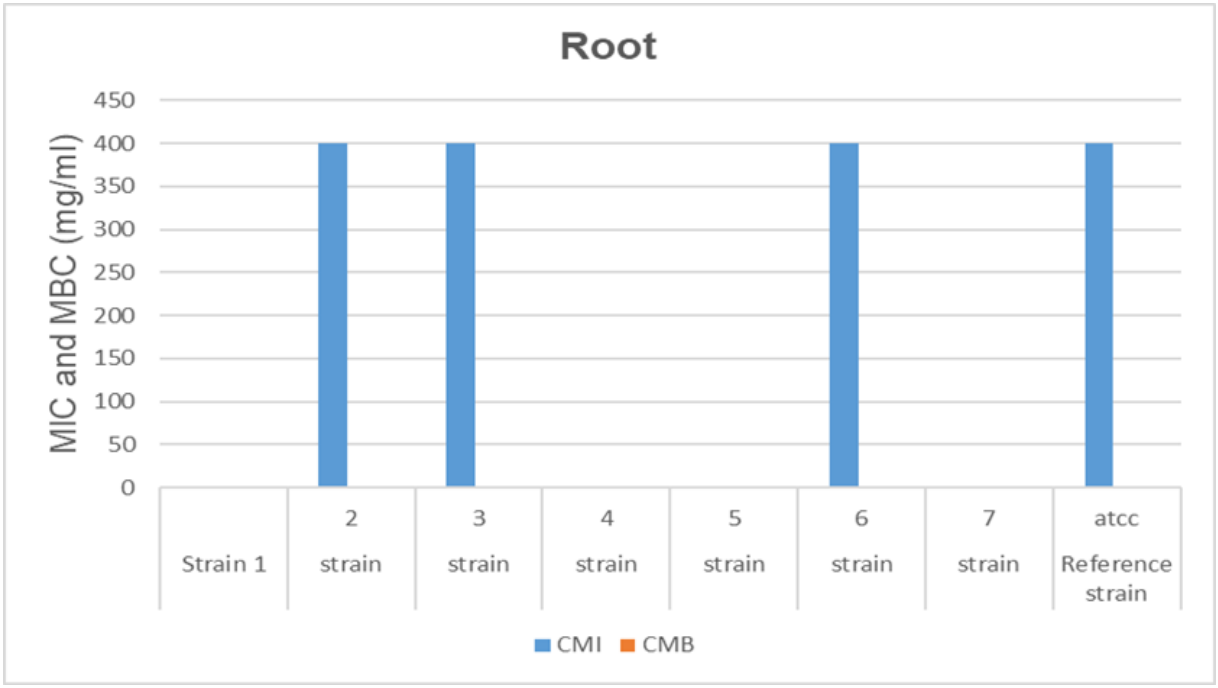


Figure 29 : MIC and MBC values of roots.

V.7. Discussion

The extraction yield of leaves (17%) was similar to the result reported by Zhang and al, (2015) (18.2%), who used 70% ethanol in a hydro-alcoholic maceration method to extract bioactive compounds from E. japonica leaves, while our results of stem (11.35 %) and root (9.65%) less than in leaves. Karam *and al*, (2023) confirm that leaves contain a higher yield. These findings support the use of leaves for optimal extraction and improved biological activity.

The observed concentrations of polyphenol content in the different organs (leaves, stems and roots) were expressed differently, this is probably due to variations in the physiological roles and environmental exposure of each organ. The higher concentration of polyphenol plant stems compared to leaves can be influenced by several factors. The stems provide support for the plant, connecting the roots to the leaves, flowers, and fruits, ensuring transport of water and nutrients **(Taiz and *al.*, 2015)**. This essential function exposes them to various mechanical and abiotic stresses, such as physical strain, pathogen attacks, and climatic variations **(Gong, 2021)**. To defend against these aggressions, the stems accumulate a higher concentration of polyphenols, notably lignins and tannins, that acting as antioxidants during transport, reinforce the rigidity of the cell walls, limit the penetration of pathogens, and act as repellents to herbivores.,, as shown by **(Mokdad-Bzeouich and *al.,* 2015)**. The leaves, exposed to UV radiation and reactive oxygen species (ROS), produce antioxidants, which explains their moderate polyphenol content like alkaloids and phenols, consistent with the findings of **(Pawlowska and *al.*, 2023)** and **(Xu and *al*., 2021)**. Although roots are less exposed to oxidative stress, they still contain specific phenolics such as triterpens and saponins involved in rhizospheric interactions, highlighting their ecological importance **(Xu and *al.*, 2021)**.

The FTIR-ATR analysis of the different parts of *Eriobotrya japonica* Lindl revealed several functional groups, reflecting the chemical richness of this plant. The leaves may contain flavonoids, which are known to protect plants from UV radiation **(Jitendra and *al*., 2024).** The presence of these compounds was also confirmed through (HPLC NMR and MS) analyses in a previous study of Xu and al. (2023), which validated the importance of *Eriobotrya japonica* Lindl leaves

as a natural source of bioactive compounds with antidiabetic potential. and We found tannins known for their role in serve as chemical defense **(Barbehenn and Constabel, 2011)** ,Through (HPLC) analysis, Izabela Nawrot-Hadzik and *al.*(2017) identified a wide range of antioxidant compounds in the leaves of *E. japonica*, including tannins, in our work we founded the Phenols are known to protect the plant from oxidative stress, UV radiation **(Ahmad and Bajguz , 2024) ,** The presence of phenolic compounds was confirmed by Seon and Soon.(2021) through HPLC DAD, being among the major constituents identified for their antioxidant potential. And also are noted alkaloids act as natural toxins to inhibe insects and parasites **(Zaynab and Shari, 2024)**.

In our study, ATR-FTIR analysis detected the presence of phenolic compounds such us lignin in the stem in agreement with Zhang and *al*. (2016) the methods of qRT-PCR and histochemical data Identified lignin accumulation in *E. japonica* stems, This compound plays a key structural and protective role and is also involved in facilitating water transport, as noted by Kankaanpää and *al*. (2023). In this research, we founded the roots of Eriobotrya japonica contain a variety of secondary metabolites, including various bioactive compounds. Among these, terpenoids such as saponins which influence seed development **(Kaure and *al*., 2024),** and triterpenes known for their role in protecting plants from pathogens **(Wang and *al*., 2024)**, were observed. Zhou and al. (2021), using UHPLC-QTOF-MS analysis, also identified a rich diversity of terpenoids in the roots. In addition, phenolic compounds such as coumarins which offer protection against insects, bacteria, and fungi **(Wu and Zhang, 2022)** were noted. We also identified the presence of other bioactive molecules, including carboxylic acids, which contribute to plant defense mechanisms against pathogens **(Singh and Jha, 2016).**

The biochemical results suggest the presence of active compounds that may confer antimicrobial activity.

Macroscopic appearance of pink to red colonies on MacConkey agar is a typical characteristic of lactose-fermenting bacteria, particularly *E.coli.* The fermentation of lactose leads to the production of acidic byproducts, which lower the pH of the medium.

This triggers the color change of the neutral red pH indicator included in the agar, resulting in the characteristic pink/red coloration **(Feng and *al.*, 2022).**

The Gram staining results are consistent with the typical morphology of *apec*, which appears as small, Gram-negative, non-spore-forming rods **(Madigan and *al.,* 2021)**. Their pink color after staining is due to a thin peptidoglycan layer and the presence of an outer membrane rich in lipopolysaccharides, a hallmark of Gram-negative bacteria **(Madigan and *al.,* 2023).** The arrangement in singles or short chains reflects non-aggregative, facultative anaerobic behavior **(Yamamoto and *al.*, 2022).** These features are characteristic of the Enterobacteriaceae family and are essential for differentiating *E. coli* from other Gram-negative bacilli **(Todar, 2021).**

The results of antibiogram showed that all the strains tested were sensitive to cefotaxime, indicating that this β-lactamine remains effective against apec, this leads to lysis of the bacterial cell, a rapid bactericidal effect, especially in Gram-negative bacteria such as E. coli **(Dubley and *al.,* 1982)**. Unlike antibiotics such as tetracycline or co-trimoxazole, which are frequently used in poultry production **(Johnson and *al.*, 2017)**, cefotaxime is rarely used in animals (often reserved for human use) **(FDA, 2012)**, study Antimicrobial resistance in clinical E. coli isolates from poultry... China Highlights low frequency of resistance to 3^{e} generation cephalosporins (ceftriaxone, cefotaxime).

On the oder hand strains ATCC, 2, 4 and 6 (4 days old) were sensitive to gentamicin due to recent microbiological age, low antibiotic exposure, absence of resistance mechanisms **(Oakley and *al*., 2014)**, while 1, 3, 5 and 7 were intermediate probably due to physiological changes (slower metabolism due to environmental stress, biofilm which is a natural barrier that reduces antibiotic penetration) and progressive genetic modifications (expression of efflux pumps which expel the antibiotic from the cell, inactivating enzymes) These adaptations enable strains to reduce antibiotic efficacy without reaching a full level of resistance **(Li and Nikaido, 2015)**, Mah and O'Toole **(2001)** explain that more mature biofilms have a slower metabolism and increased physical barriers, reducing the penetration of antibiotics such as gentamicin.

All APEC strains are resistant to tetracycline and co-trimoxazole, probably due to their frequent use on poultry farms and the acquisition of plasmid resistance genes, **(Van and *al.,* 2008)** confirm that avian E. coli are highly resistant to tetracycline (87%)

and co-trimoxazole (72%), **(Nguyen and Nguyen, 2016)** highlighted tetA, tetB, sul1, sul2, dfrA1 genes, responsible for resistance to tetracycline and co-trimoxazole.

The reference strain Escherichia coli ATCC 25922 has not developed antibiotic resistance mechanisms, as it is a standardized, “clean,” and well characterized strain used as a quality control in laboratories **(CLSI, 2023).** The overuse and misuse of antibiotics in poultry production can contribute to the selection of antibiotic resistant bacteria and the spread of resistance genes. , the specific virulence factors and antibiotic resistance genes present in APEC strains can vary, thereby influencing their sensitivity to different antibiotics **(Kovács and *al.*, 2024).**

The prolonged and excessive use of antibiotics, particularly in poultry farming, is leading to the emergence of resistant bacterial strains, significantly reducing the effectiveness of conventional treatments in both animals and humans. This situation raises a major public health concern, as some strains of avian pathogenic Escherichia coli (APEC) can be transmitted to humans through the consumption of contaminated poultry meat. In this context, the use of plant extracts with antibacterial activity, such as those of Eriobotrya japonica, represents a promising natural alternative. This positioning reinforces the interest of these extracts as an alternative solution in the event of human infection by resistant zoonotic pathogens from the food chain **(Abdou and *al.*, 2011).**

The results obtained show that *E. japonica* extracts exhibit variable antibacterial activity depending on the organ used for the extract (leaves, stems, roots), their different concentrations and the age of broiler chickens were used for the isolation of apec.
Dual inhibitory and bactericidal action of leaves, may be related to their richness on bioactive compounds. younger strains 2, 6 and ATCC generally showing greater sensitivity to the extract while older strains (1, 5 and 7 of 21) days exhibited reduced responsiveness.The stem extract showed both inhibitory and bactericidal activity against most strains aged 4 days old (2, 6 and ATCC), On the other hand, it exerted essentially inhibitory activity, with no notable bactericidal effect against strains aged 21 days old (1, 5 and 7). The root extract exhibited showed only an inhibitory effect for most strains aged 4 days old (2, 6, and ATCC), and no effect for strains aged 21 day old. the lower richness in antibacterial compounds probably related with their roles of

support and transport of nutrients **(He and *al*., 2023).** Abdou and *al*.(2011**)**demonstrate that the ethanolic extract of eriobotrya japonica leaves better inhibits the growth of ESBL-E. coli and K. pneumoniae strains, and stems extract is less effective. antibacterial activity correlates with plant extracts concentrations.

The higher sensitivity of 4 day old strains may be due to the immature immune system and underdeveloped gut microbiota of young chicks, making them more vulnerable to antibacterial agents. Limited exposure to antibiotics and environmental stressors further reduces resistance. In contrast, older birds often carry more resistant bacteria due to prior exposure to selective pressures.

The ATCC reference strain is a well characterized laboratory strain that has not been exposed to environmental or antibiotic selection pressures, unlike field strains. As a result, it tends to maintain high sensitivity to antibacterial agents. This explains why it responded similarly to the 4 day old strains, Hjerde and *al.,* (2017) demonstrate that "wild-type" or unexposed laboratory strains maintain greater sensitivity (less resistance) than induced clinical strains or strains isolated in hospitals.

despite strains (2, 4 and 6) derived from chicks aged just 4 days, strain 4 in particular showed similar behavior to strains (1, 5 and 7), all derived from 21 day old birds. This suggests that the age of the animal is not the only factor determining sensitivity to extracts. In contrast, strain 3 although isolated from a 21 day old bird, showed a high level of sensitivity comparable to that of younger strains.

These observations can be attributed to farming conditions such as feeding, hygiene, population density, ventilation and antibiotic exposure strongly influence microbial composition and bacterial resistance **(De Vos and *al.*, 2023)**.

Conclusion

The growing emergence of multidrug-resistant bacteria represents a major challenge to both animal and human health. In this context, the exploration of natural sources with antimicrobial potential particularly medicinal plants has emerged as a promising alternative to conventional therapies. This study was conducted with that objective in mind: to evaluate the antibacterial potential of hydroalcoholic extracts from different parts (leaves, stems, roots) of *Eriobotrya japonica* Lindl. against several strains of Avian Pathogenic Escherichia coli (APEC), a bacterium that causes significant economic losses in the poultry industry and poses a serious zoonotic threat to public health.

The results revealed a moderate antibacterial activity, most notably in leaf extracts, which were found to be particularly rich in phenolic compounds. This antibacterial efficacy gradually decreased from leaves to stems and finally to roots, a trend consistent with the phytochemical analyses (total phenolic content and FTIR-ATR spectra) that confirmed a higher concentration of bioactive compounds in the leaves. The experimental data showed that the leaves yielded the highest extraction percentage (17%), followed by stems (11.35%) and roots (9.65%). The phenolic content was also significantly higher in the leaf extracts, as confirmed by spectroscopic analysis. Antibacterial tests using the disk diffusion method, MIC, and MBC evaluations demonstrated that leaf extracts had the strongest antibacterial effect, indicated by the largest inhibition zones and the lowest MIC and MBC values across all tested APEC strains, including both reference and field isolates. This activity consistently decreased in stem and root extracts, correlating with their lower phenolic content.

Although E. japonica cannot currently replace antibiotics in treating APEC infections, it shows promising potential as a complementary agent in the fight against antibiotic-resistant bacteria. The interest in this plant lies in its abundance of secondary metabolites such as flavonoids, tannins, and phenolic acids known for their antimicrobial, antioxidant, and anti-inflammatory properties. These compounds may act through multiple mechanisms, including inhibition of bacterial virulence, disruption of biofilm formation, and interference with essential metabolic enzymes. As such, incorporating E. japonica extracts alongside existing treatments could reduce the bacterial burden in infected poultry and mitigate the selection pressure that drives resistance development.

However, it is important to recognize the limitations of this study. The findings are preliminary and based solely on in vitro experiments. Furthermore, the absence of toxicological assessments, compound isolation, or in vivo validation precludes any immediate application in veterinary or human medicine. Therefore, further research is essential, particularly studies aimed at identifying the specific active constituents, confirming their safety, and elucidating their mechanisms of action.

This work also contributes to broader public health strategies focused on food safety and sustainable antimicrobial use. By potentially lowering the bacterial load in poultry, E. japonica could help reduce the risk of transmitting resistant strains to humans via the food chain. This aligns with the “One Health” approach, which emphasizes the interconnectedness of human, animal, and environmental health in managing infectious disease risks.

In conclusion, while *Eriobotrya japonica* lindl cannot yet be considered a therapeutic solution for APEC infections in poultry, its potential as a natural adjunct or preventive agent in antimicrobial stewardship programs is worthy of further exploration. This thesis thus opens the door to future multidisciplinary investigations spanning phytopharmacology, microbiology, toxicology, and clinical research toward the development of safe, effective, and environmentally responsible therapeutic alternatives.

Annex1: Composition of medias

Composition of Nutrient Agar (in g/L)

Peptone 5g

Meat extract. 01g

Yeast extract. 02

Sodium chloride 05g

Agar. 15g

PH = 7,4

Composition of Mueller-Hinton Agar (in g/L)

Beef infusion 300cm3

Casein peptone (acid hydrolysate of casein)..... 17.5g

Corn starch… 1.5g

Agar. ..... 17 g

PH = 7,4

Composition of Mueller-Hinton Broth (g/L)

Beef infusion ..... 1 Litre

Casein peptone (acid hydrolysate of casein)..... 59g

Starch… 5g

pH=7,4

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الجمهورية الجزائرية الديموقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

جامعة عبد الحميد بن باديس-مستغانم-
كلية علوم الطبيعة و الحياة

تصريح شرفي خاص بالالتزام بقواعد النزاهة العلمية
لإنجاز البحث

أنا الممضي أدناه،

الطالب(ة): بومعزة مباركية رقم التسجيل الجامعي: 202037028417

الحامل لبطاقة التعريف الوطنية رقم: 108373714 والصادرة بتاريخ: 03/2022

عن بلدية مستغانم

المسجل ب كلية علوم الطبيعة والحياة / قسم البيولوجيا

شعبة علم السموم / التخصص علم الصيدلة والسموم

والمكلف بإنجاز مذكرة ماستر بعنوان:

Anti-APEC Activities of Eriobotrya japonica Lindl extracts (Leaves, Stems and Roots)

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات العلمية والنزاهة الأكاديمية المطلوبة في إنجاز البحث، وأتحمل المسؤولية الشخصية عن كل المحتوى المتضمن في البحث المذكور أعلاه .

التاريخ: 2025/06/11

إمضاء المعني

* ملحق القرار الوزاري رقم 933 المؤرخ في 28 جويلية 2016 الذي يحدد القواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.

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أنا الممضي أدناه،

الطالب(ة): لعريبي أسية زينب ..... رقم التسجيل الجامعي: 202037022142

الحامل لبطاقة التعريف الوطنية رقم: 141782792 والصادرة بتاريخ: 2024-03-24

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