الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة سعيدة الدكتور مولاي الطاهر

Dr. Moulay Tahar University of Saïda

كلية علوم الطبيعة والحياة

Faculty of Nature and Life Sciences

قسم البيولوجيا

N° d'Ordre

Department of Biology

A Thesis Submitted for the Degree of Master

In Biological Sciences

Specialty: Biochemistry

Theme

Phytochemicals Screening and Bioactivities of Green Tea Extracts (infusion, decoction, ethanolic) Marketed in the Wilaya of Saida

Presented by:

- Miss Rania ZOUBIR

Defended on: 23/06/2026

Before the Jury Composed of :

President	Mr. Khaled KAHLOULA	Pr UMTS University
Examiner	Mrs. Amel SIDI IKHLEF	MCB UMTS University
Supervisor	Mrs. Dalila GHOUTI	MCA UMTS University

Academic Year: 2025 – 2026

Acknowledgements :

Praise be to Allah, the Most Gracious, the Most Merciful, for the strength, patience, and guidance He granted me throughout this work. It is by His grace and blessings that I was able to overcome the challenges and successfully complete this thesis

I express my deepest gratitude and appreciation to my supervisor Mrs Dalila Ghouti, for accepting to supervise this work. I am profoundly grateful for her patience, her wise advice, her valuable guidance, and the great trust she placed in me throughout this research.

My sincere appreciation goes to President Mr. Khaled Kahloula and Examiner Mrs. Amel Sidi Ikhlif for accepting to evaluate this thesis as members of the defense committee, and for the quality of their scientific remarks.

I am also very grateful to the Head of the Laboratory at the University of Saïda, for his/her welcoming support and guidance.

I would also like to warmly thank the entire staff of the Biochemistry and Microbiology laboratories at the Faculty of Nature and Life Sciences, Dr. Moulay Tahar University of Saïda, for their accompaniment, guidance, and technical assistance throughout my laboratory experiments.

Finally, I extend my heartfelt gratitude to all the people—family, friends, and colleagues—who supported, encouraged, and motivated me, from near or far, in the realization of this modest work.

Dedication

To my dear Mother:

My partner in this journey and my source of love... Thank you for your endless support, and thank you for every sacrifice you made just to see me happy and confident. I love you so much. You will always be my warm comfort, and I promise you that I will always make you proud, with Insha'Allah.

To my beloved Father:

My strong pillar and my guide... I thank you from the bottom of my heart for everything you gave me throughout my long years of study. Thank you for your great efforts to help me become a successful and educated woman.

To my sweet sister :

My number one supporter and my permanent strength. Thank you for every single moment you stood by my side, and for your help during the hardest times. Thank you for always encouraging me and believing in me.

To my dear brother:

My strength and my pride... Thank you for your financial and emotional support, for believing in my abilities, and for your deep trust in me. I am so lucky to have you in my life. Thank you for your patience and for everything you have done for me.

To my loyal friend, "Maroua":

My companion... Thank you for supporting me all this time, for helping me with this research work, and for always keeping me motivated. Thank you for all the beautiful memories we created together during this journey.

To all my friends:

To those who shared the classroom and the days with me... Thank you for your support during this period, for the wonderful memories, and for every difficult day we passed together side by side. I thank God for bringing you into my life.

To everyone who helped me:

To every person who lent a helping hand in this research and helped make it successful... Thank you so much for everything you provided.

List of Abbreviations :

- **%:** Percent sign.
- **$A_{0,5}$:**Absorbance at 0.5
- **ABTS:** 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).
- ***C. sinensis*:** *Camellia sinensis.*
- **CFU:** Colony-Forming Unit
- **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl.
- **$FeCl_3$** :Ferric Chloride.
- **FRAP:** Ferric Reducing Antioxidant Power.
- **HAT**: Hydrogen Atom Transfer.
- **IC_{50}:** Half-Maximal Inhibitory Concentration .
- **K_3 Fe$(CN)_6$:** The potassium ferricyanure.
- **LAPRONA**: Laboratory of Natural Products (University of Tlemcen);
- **MBC:** Minimum Bactericidal Concentration.
- **mg:** Milligram.
- **MIC:** Minimum Inhibitory Concentration .
- **mm:** Millimeter.
- **MNHN**: National Museum of Natural History (Paris).
- **OD:** Optical Density.
- **PBS:** Phosphate Buffered Saline.
- **ROS:** Reactive Oxygen Species.
- **SET**: Single Electron Transfer.
- **TCA:** Trichloroacetic Acid.
- **UV-Vis:** Ultraviolet-Visible (Spectrophotometry).
- **µg:** Microgram.
- **µl:** Microliter.

- **List of Tables:**

- **List of Figures:**

Abstract:

This study evaluates the biological activities of three extracts (infusion, aqueous and ethanolic decoction) from *Camellia sinensis* (green tea) leaves, using two commercial brands consumed in Saida: "El-Berrad" and "El-Raqim". Phytochemical screening revealed an abundance of flavonoids, tannins, alkaloids, and sterols, a weak presence of coumarins and saponins, and a total absence of anthocyanins.

The ethanolic extracts demonstrated remarkable DPPH radical scavenging activity with IC_{50} values of 0.014 mg/ml for "El-Berrad" and 0.015 mg/ml for "El-Raqim", followed by aqueous decoctions (IC_{50} = 0.16 and 0.12 mg/ml respectively), while the hot infusion showed the lowest activity. For the FRAP assay, the ethanolic extract of "El-Raqim" was the most promising (IC_{50} = 0.036 mg/ml).

Anti-inflammatory activity (HRBC assay) showed maximum membrane stabilization of 68.14% for "El-Berrad" and 69.03% for "El-Raqim" at the highest concentration.

The antimicrobial assay via well diffusion targeted four bacterial strains *(Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus)* and two fungal strains *(Penicillium zacinthae, Aspergillus niger)*. The ethanolic extract had the greatest effect against *Staphylococcus aureus* (inhibition zones of 6–16 mm for "El-Berrad" and 6–15 mm for "El-Raqim") with a MIC of 1.09 mg/ml for both brands. No activity was recorded against *Escherichia coli* or the fungal strains.

Finally, the MBC test demonstrated a bacteriostatic rather than a bactericidal effect. In conclusion, these results confirm a high biological convergence between both brands and suggest that reflux decoction optimizes bioactive compound extraction compared to the infusion method.

- **Keywords**: Green tea (Camellia sinensis), El-Berrad, El-Raqim, Antioxidant activity, Anti-inflammatory activity, antimicrobial activity, IC_{50} .

Résumé :

Cette étude évalue les activités biologiques de trois extraits (infusion, décoction aqueuse et éthanolique) des feuilles de *Camellia sinensis* (thé vert) à travers deux marques consommées à Saïda : « El-Berrad » et « El-Raqim ». Le criblage phytochimique a révélé la richesse des extraits en flavonoïdes (Flavonoids), tannins (Tannins), alcaloïdes (Alkaloids) et stérols (Sterols), une faible présence de coumarines (Coumarins) et saponines (Saponins), et l'absence totale d'anthocyanes (Anthocyanins).

Les extraits éthanoliques ont montré une excellente activité antioxydante (DPPH) avec des IC_{50} de 0,014 mg/ml pour « El-Berrad » et 0,015 mg/ml pour « El-Raqim », suivis des décoctés aqueux (IC_{50} = 0,16 et 0,12 mg/ml respectivement), alors que l'infusion a affiché le pouvoir le plus faible. Pour le test FRAP, l'extrait éthanolique de « El-Raqim » s'est révélé le plus prometteur (IC_{50} = 0,036 mg/ml).

L'activité anti-inflammatoire (test HRBC) a atteint un taux maximal de stabilisation membranaire de 68,14% pour « El-Berrad » et 69,03% pour « El-Raqim »

L'évaluation antimicrobienne par diffusion sur gélose a ciblé quatre souches bactériennes *(Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus)* et deux souches fongiques *(Penicillium zacinthae, Aspergillus niger)*. L'extrait éthanolique a montré l'effet le plus important contre *Staphylococcus aureus* (zones d'inhibition de 6-16 mm pour « El-Berrad » et 6-15 mm pour « El-Raqim ») avec une CMI de 1,09 mg/ml pour les deux marques. Aucun effet n'a été enregistré contre *Escherichia coli* ni contre les souches fongiques.

Enfin, le test de la CMB a démontré un effet bactériostatique plutôt que bactéricide. Ces résultats confirment la forte convergence biologique entre les deux marques et prouvent que la décoction à reflux optimise l'extraction des composés bioactifs par rapport à l'infusion.

Mots-clés : Thé vert (*Camellia sinensis*), El-Berrad, El-Raqim, Activité antioxydante, Activité anti-inflammatoire, Activité antimicrobienne, IC_{50}.

الملخص:

تستهدف هذه الدراسة تقييم الأنشطة البيولوجية لثلاثة مستخلصات (نقع ، مغلى مائي، ومغلى إيثانولي) لأوراق نبات *Camellia sinensis* (الشاي الأخضر) لعلامتين تجاريتين الأكثر استهلاكاً في منطقة سعيدة: «البراد» و«الرقيم». كشف الفرز الفيتوكيميائي عن غنى المستخلصات بالفلافونويدات (Flavonoids)، العفص (Tannins)، القلويدات (Alkaloids)، والستيرولات (Sterols)، مع حضور ضئيل للكومارينات (Coumarins) والسابونينات (Saponins)، وغياب تام للأنتوسيانينات (Anthocyanins).

أظهرت المستخلصات الإيثانولية تفوقاً ملحوظاً في تثبيط الجذور الحرة DPPH بقيم IC_{50} بلغت 0.014 ملغ/مل لعلامة «البراد» و0.015 ملغ/مل لعلامة «الرقيم»، متبوعة بالمستخلصات المائية المغلاة (IC_{50}= 0.16) و0.12 ملغ/مل على التوالي)، بينما سجل النقع الحار أدنى نشاط مضاد للأكسدة. في اختبار FRAP، كان المستخلص الإيثانولي لعلامة «الرقيم» الأكثر واعدية (IC_{50} = 0.036 ملغ/مل).

كما كشف اختبار تثبيط غشاء كرات الدم الحمراء (HRBC) عن قدرة مضادة للالتهابات بنسبة استقرار بلغت 68.14% لعلامة «البراد» و69.03% لعلامة «الرقيم» عند أعلى تركيز.

بالنسبة للنشاط المضاد للميكروبات بطريقة الانتشار في الآبار، فقد استهدف أربع سلالات بكتيرية (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus*) وسلالتين فطريتين (*Penicillium zacinthae, Aspergillus niger*). أظهر المستخلص الإيثانولي التأثير الأكبر تجاه بكتيريا *Staphylococcus aureus* (بأقطار هالات تثبيط 6-16 مم لعلامة «البراد» و6-15 مم لعلامة «الرقيم») بتركيز مثبط أدنى (MIC) قدره 1.09 ملغ/مل لكلا العلامتين، في حين انعدم التأثير تماماً على بكتيريا *Escherichia coli* وجميع السلالات الفطرية المختبرة.

أخيراً، أثبت اختبار التركيز الأدنى القاتل للبكتيريا (MBC) أن للمستخلصات تأثيراً كابحاً للبكتيريا (Bactériostatique) وليس قاتلاً. تؤكد هذه النتائج التقارب البيولوجي الكبير بين العلامتين، وتثبت أن طريقة المغلى المرتد (Decoction) هي الأكثر كفاءة في استخلاص المركبات النشطة بيولوجياً مقارنة بالنقع.

- **الكلمات المفتاحية:** الشاي الأخضر (*Camellia sinensis*)، البراد، الرقيم، مضادات الأكسدة، مضادات الالتهاب، مضادات الميكروبات، IC_{50}.

Contents:

Introduction

Introduction:

Tea is the second most consumed beverage globally after water, highly valued for its health-promoting properties **(Brimson, J.M *et al*.2023).** Its therapeutic use dates back to 3000 BC in ancient China, as documented in Shen Nong's Herbal Classic. Today, tea consumption spans over 160 countries **(Radeva-Ilieva *et al*.2025)**. In Algeria, particularly in the Great South, tea holds an exceptional cultural status as the most popular traditional beverage consumed daily. Botanically, tea belongs to the *Theaceae* family, with *Camellia sinensis* (L.) Kuntze being the primary species utilized **(Prasanth, M.I *et al*.2019).** The main commercial varieties are derived from two distinct cultivars: Camellia sinensis var. sinensis (small-leaved) and *Camellia sinensis var. assamica* (large-leaved) **(Brimson, J.M *et al*.2023).**

The plant tissues of *C. sinensis* possess a complex chemical profile rich in bioactive substances (BAS), including polyphenols, alkaloids, saponins, polysaccharides, fatty acids, vitamins, and minerals **(Prasanth, M.I *et al*., 2019) (Xing, L. *et al*. 2019)**. The biological efficacy of green tea is primarily attributed to its polyphenolic fraction, specifically catechins **(Radeva-Ilieva *et al*.2025).** According to the EFSA, green tea yields approximately 126 mg of total catechins per 100 mL, while the FDA notes that its most potent catechin, epigallocatechin gallate (EGCG), averages 71 mg per 100 mL. In contrast, black tea contains 200 mg of flavonoids per 100 mL due to enzymatic oxidation during processing **(Rietveld A *et al*. 2003).**

Historically esteemed in traditional Asian medicine, green tea has recently gained immense global popularity due to its unique organoleptic profile and pleasant aroma. Numerous in vitro and in vivo studies have confirmed its broad-spectrum biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-aging, weight-regulating, and anticarcinogenic effects **(Truong V.L *et al*. 2021; Wong M *et al*. 2022).** Consequently, tea is classified as a functional food that provides significant physiological benefits beyond basic nutrition , acting as a natural free radical scavenger that protects the cardiovascular and central nervous systems against chronic and neurodegenerative diseases **(Yang, C.S *et al*., 2013).**

In conclusion, the chemical composition of green tea is dynamic and heavily influenced by environmental and technical factors. These include soil conditions, climate, seasonal harvesting, post-harvest processing (such as steaming or drying), and storage conditions **(Radeva-Ilieva *et al*.2025)**. These quantitative and qualitative variations directly alter the organoleptic properties (color, aroma, and taste) of the final infusion **(Krahe J *et al*. 2021; Wakamatsu M *et al*. 2019**). Furthermore, the geographical origin whether Chinese, Japanese,

or Korean plays a critical role in determining the specific chemical profile of the bioactive compounds targeted for analysis **(Koch, W *et al*. 2018; Candela L *et al*. 2020).**

Within this framework, the objective of this study is to perform a phytochemical screening to detect the active chemical groups and bioactive molecules in the leaves of *Camellia sinensis* (green tea) available in the Algerian market. Specifically, this research focuses on studying and comparing two commercial brands: "El-Berrad" and "El-Raqim", as they are the two most consumed and popular brands by people in the Saïda region due to their daily use.

Furthermore, this study aims to verify the therapeutic efficacy and promote the value of this medicinal plant by evaluating its biological activities *in vitro*. These evaluations include testing its antioxidant, anti-inflammatory, and antimicrobial properties. To achieve these goals, a detailed scientific study was conducted by preparing three different extracts from both brands ("El-Berrad" and "El-Raqim"). Two solvents were used: ethanol and distilled water, applying three different extraction methods: Decoction (reflux), and infusion. This approach allows us to confirm the biological effects of the most popular tea brands in the region.

Part one:

Bibliographic Study.

Chapter I :
Bibliographic Review on Green Tea (Camellia sinensis)

I.1 History and Origin of Tea :

At least 30 countries cultivate tea currently, which has been a popular medicinal plant utilized in countries such as China and India for centuries. There are many different traditional health care systems, including Chinese traditional medicine, Ayurveda medicine, and Himalayan medicine, all of which utilize tea in their philosophies. For thousands of years, tea has been consumed throughout numerous Asian nations, including, but not limited to, India, China, Japan, and Thailand **(Parmar Namita *et al*, 2012)**

The true origin of tea is not known definitively, but some researchers speculate it originated in Assam, India, while other people theorize it began in southern China. It has been said that the first person to drink tea was a man called (Shen Nung), or as some claim, Emperor (Shien Non Shei), who accidentally discovered it around 2700 BC (**Rishi Raj Shrivastava *et al.* ,2018).**

In less than 20 years of being introduced to China, green tea had become a popular drink among the wealthy elite in China. John states in his book of (Tea -its history and mystery) that although tea was first cultivated and consumed throughout Asia (including countries like India and eventually Japan) over thousands of years, there is a direct connection linking tea with the country of China. For example, John identifies multiple ancient references to tea (such as the "Sao pao" from around 2700 BCE), and although the oldest known reference of tea is from around 600 BCE ("Ray"), no definitive record exists to confirm when or where the first cup of tea was made. According to John, almost 2,000 years ago, tea was already cultivated and added to the vast number of plants now classified as tea growing around the world; however, the purpose of its cultivation was to promote friendship and goodwill between the East's kings/rulers at that time and to solidify the bond between food and health. **(Joseph M. Walsh . 1892).**

I.2 Botanical Study of Camellia sinensis:

I.2.1 Definition :

Tea, which comes from the *Camellia sinensis* plant, is the second most popular drink in the world after water. This plant is a member of the genus *Camellia*, which is a group of flowering plants in the family *Theaceae*. Tea is a drink with few calories that comes in different colours, each of which stands for a different type of tea. Green tea is known for having more antioxidants than other types of tea **(Henning et *a*l., 2003).**

Camellia sinensis is the plant that all major types of tea come from. These include white, green, oolong, and black teas. However, they are processed in different ways to get different levels of oxidation. Kukicha, or twig tea, is made from the same type of plant, but it uses twigs and stems instead of leaves. Some common names for this plant are tea tree, tea plant, and tea shrub **(Rawat Mukesh et *a*l., 2012).**

I.2.2 Nomenclature and Taxonomy :

Historically, different parts of China have used different names for the tea plant, such as "Tcha," "Cha," "Tha," "Thea," and "Tscha." The European word "TEA" comes from the Fo-kien word "Tia." Linnaeus named the plant Thea, which comes from the Greek word for "goddess." There has been a long-standing debate among taxonomists about whether tea should be its own genus or belong to the genus *Camellia* **(Joseph M. Walsh. ,1892).**

The genus *Camellia* has about 82 species, most of which are native to the highlands of Southeast India. Early botanists observed variations in leaf morphology; however, subsequent scholars such as Bentham and Hooker consolidated tea and Camellia into *Thea-Camellia*, primarily differentiating them by fruit structure: tea yields a three-lobed capsule containing a single round seed per cell, whereas *Camellia* produces larger, triangular capsules. Even though tea is important to the economy, its taxonomy is still hard to understand because of how different its traits can be and how they can be similar to other plants. The historical emphasis on economically valuable varieties has diminished fundamental taxonomic research; however, comprehending genetic diversity and taxonomic characteristics is crucial for identifying superior genotypes to improve commercial tea cultivation and to distinguish authentic tea plants from ornamental *Camellia* species. **(K. C. Willson et al. ,1992).**

I.2.3 Taxonomy:

The historical dispute was ultimately settled by recognising the considerable morphological variability within the species. Modern botanists, building on the work of Bentham and Hooker and later taxonomists, have come to the conclusion that species that were previously thought to be separate genera, like Thea viridis and Thea bohea, are actually phenotypic variants of the same species. Consequently, the prevailing classification amalgamates these variants under the singular nomenclature Camellia sinensis (L.) Kuntze, acknowledging them as botanical varieties rather than distinct species. This change shows that we now have a better understanding of how the plant's genes and structure fit together in the Theaceae family.

Table 1: Scientific Classification of Tea (*Camellia sinensis).*

Taxonomic Rank	**Classification**
Kingdom	Plantae (Plants).
Subkingdom	Tracheobionta (Vascular plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Dilleniidae
Order	Ericales
Family	Theaceae (Tea family)
Genus	Camellia L
Species	***Camellia sinensis* (L.) Kuntze**

I.2.4 Botanical description *:*

Camellia sinensis, a member of the Theaceae family, is an evergreen tree or shrub that reaches a height of 10 to 15 meters in the wild and 0.6 to 1.5 meters under cultivation **(Rani R et al., 2014)**. It is commonly known as "Tea" or "Cha" **(Shrivastava RR et al., 2018).**

Figure 2 : Historical herbarium specimen of *Camellia sinensis* (1928), Harvard University Herbaria.

Figure 1 : Growth habit of an ancient tea tree (Camellia sinensis) in its natural habitat

Hojo, A. (2017).

Leaves:

- **Arrangement**: Alternate.
- **Petiole length**: 3–7 mm, pubescent and glabrescent.
- **Leaf blade**: Thinly leathery, elliptic, oblong-elliptic, or obovate-elliptic.
- **Size**: 5–12 cm long and 1.8–4.5 cm wide.
- **Apex**: Mucronate or blunt-pointed.
- **Base**: Cuneate.
- **Margin**: Serrate.
- **Surface**: Glossy dark green on the upper side, sometimes slightly hairy or glabrous; young leaves appear silver due to downy hairs on the surface.
- **Veins:** Approximately 8 pairs of lateral veins, clearly visible.

- **Additional features:** Young leaves bear short white hairs on the underside.

Figure 4: *Camellia sinensis* leaves, © **Board of Trustees of the Royal Botanic Gardens, Kew (2024).**

Figure 3: Camellia sinensis (L.) Kuntze *leaf,* Pl@ntNet,

Flowers:

- **Type**: Bisexual and fragrant.
- **Arrangement:** Solitary or in clusters of 2 to 4 in leaf axils.
- **Pedicel length**: 6–10 mm, curved downward.
- **Diameter**: 2.5–3.5 cm.
- **Sepals:** 5–6, rounded, mucilaginous with membranous margins and persistent lashes.
- **Petals**: 5–8, broadly obovate; outer petals are sepaloid while inner petals are obovate to broadly obovate.
- **Stamens:** Numerous, 0.8–1.3 cm long, with outer filaments fused into a tube.
- **Ovary**: Superior, tomentose, 3-locular.
- **Style**: Single, apically 3-lobed.
- **Color**: Yellow-whit

Figure 5*: Camellia sinensis* flowers, **© Board of Trustees of the Royal Botanic Gardens, Kew (2024).**

Branches and Buds:

- **Young branches**: Grayish yellow and glabrous..
- **Current year branchlets**: Purplish red.
- **Terminal buds:** Silvery gray and covered with silky hairs (sericeous).

Figure 6 : Morphological structure of a tea branch showing the terminal bud and young leaves **(Yoshan Tea, 2023).**

Fruits:

- **Appearance**: Brownish-green
- **Seeds**: One to four, subglobose or flattened, brown, 1–1.4 cm in diameter.

Figure 7 : External morphology of Camellia sinensis fruits © **Board of Trustees of the Royal Botanic Gardens, Kew (2024).**

Flowering and Fruiting:

- **Flowering period:** October to February.
- **Fruiting period**: August to October.

I.3 Geographical Distribution and Ecology :

The tea plant, *Camellia sinensis*, is cultivated across a vast global range, primarily between latitudes 42°N and 27°S. Its production is concentrated in over 30 countries, with major hubs in China, India, Kenya, and Sri Lanka. The plant thrives in monsoon and tropical climates characterized by high annual rainfall (minimum 1700 mm) and strictly requires acidic soils (pH 4.5–5.6). Environmental factors such as elevation (up to 2300 m) significantly influence the synthesis of secondary metabolites, where high-altitude environments are known to enhance the aromatic quality and chemical complexity of the leaves **(Schoorel & van der Vossen, 2000; Wang et al., 2018).**

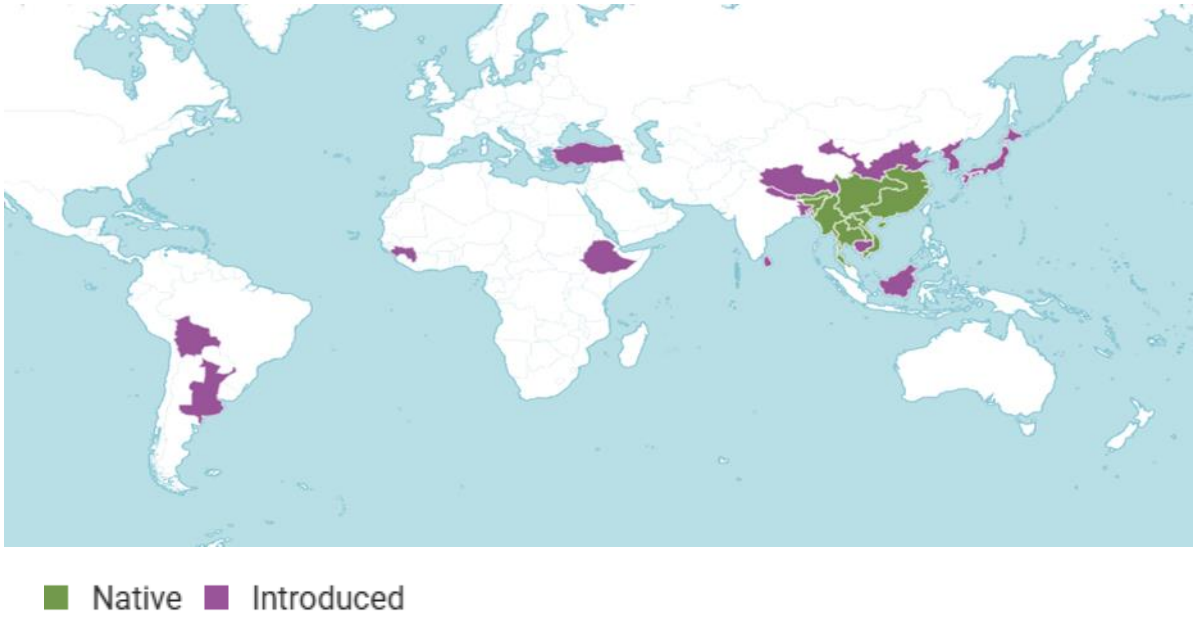


Figure 8 : Native world distribution map of *Camellia sinensis* **© Board of Trustees of the Royal Botanic Gardens, Kew (2024).**

I.3.1 Botanical Varieties: A Comparative Overview :

The tea industry distinguishes between two primary botanical varieties that offer a balanced diversity in morphology and biochemistry **(Agarwal et al., 2017):**

The China Variety (*var. sinensis*): Indigenous to temperate regions, this variety features small, narrow leaves and high resistance to cold and frost. It is predominantly used for producing green teas due to its delicate flavor profile and specific catechin composition.

The Assam Variety (*var. assamica*): Native to the tropical forests of Northeast India, it possesses much larger, broader leaves and thrives in humid, low-elevation environments. It is characterized by a higher content of polyphenols and caffeine, making it the ideal choice for black tea production.

I.4 Cultivation and Biochemical Processing

The chemical transformation of tea is governed by the rigorous management of endogenous enzymes, moisture levels, and thermal treatments:

Harvesting and Cultural Practices: Tea shoots are harvested at 4 to 14-day intervals, depending on the growth rate and season. Cultivation typically begins after a 2 to 4-year immaturity period, during which plants are maintained as shrubs (1–1.2 m height) through regular pruning to maximize yield and facilitate manual or mechanical plucking **(Martin et al., 1997).**

Figure 9 : Manual harvesting of tea shoots **(Planted Well; 2022), (POWO; 2024).**

Withering and Spreading: Upon harvest, leaves containing 75–83% moisture are withered to reduce water content to less than 70%. In green tea production, this step is minimal (or replaced by post-harvest spreading for 1–3 hours) to prevent early metabolic degradation and preserve the fresh biochemical state **(Ye et al., 2022).**

Figure 10 : Withering process to reduce leaf moisture content **(Planted Well; 2022).**

Enzymatic Control (Fixation): The core of tea biochemistry lies in the management of polyphenol oxidase (PPO), along with other key enzymes such as catalase, peroxidase, and ascorbic acid oxidase. For green tea, immediate heat treatment (Fixation) at temperatures ranging from 100°C to 200°C (pan-firing) or approximately 100°C (steaming) is applied to inactivate these enzymes. This halting of enzymatic pathways prevents the oxidation of catechins and maintains the characteristic green color **(Subramanian et al., 1999; Chen et al., 2019).**

Figure 11: Thermal inactivation of Polyphenol Oxidase (PPO) via Fixation) **(Planted Well; 2022)**..

Rolling and Chemical Stabilization: Following fixation, leaves are rolled to disrupt cell walls and release intracellular constituents. This process facilitates the hydrolysis of proteins into free amino acids (aroma precursors) and enhances the extractability of tea solids. Finally, drying stabilizes the product for storage. It is noteworthy that biochemical stability remains a challenge; for instance, the primary antioxidant EGCG can degrade by up to 28% within six months of storage if not kept in optimal conditions **(Selena Ahmed *et a*l.2013).**

Figure 12: Final processing stages: (A) Mechanical rolling, (B) Stabilization by drying) **(Planted Well; 2022).**

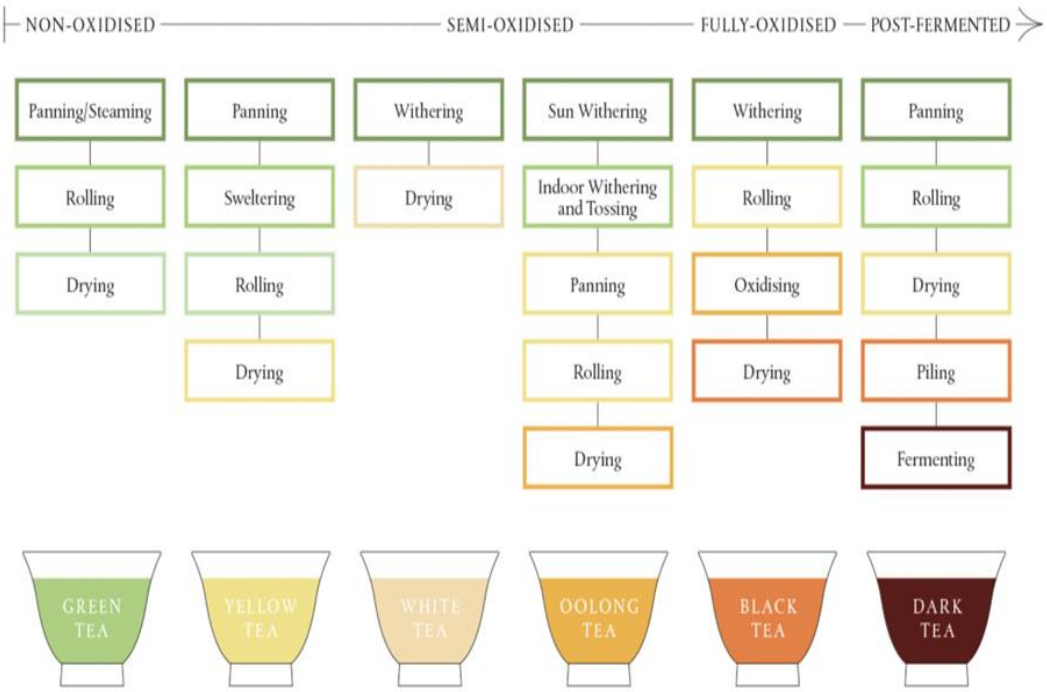


Figure 13: Classification of tea types based on oxidation levels and specific processing pathways. **(Wikifarmer, 2023)**

I.5 Uses and Potential Applications of Tea

Beyond its botanical classification, green tea (Camellia sinensis) possesses significant industrial utility. In the food industry, it is widely employed as a natural additive to improve the viscoelastic properties of dough and to protect various meat products from lipid oxidation. Furthermore, its rich antioxidant profile has led to its extensive incorporation into the cosmetics industry, where it serves as a key ingredient in UV protection formulas (sunscreens) and dermatological cleansers **(Cheng Wang *et al.*2022).**

From a pharmacological perspective, traditional medicine has long utilized green tea extracts as a diuretic and an effective astringent for wound healing. It is also traditionally administered to treat gastrointestinal distress, flatulence, and regulate blood sugar. Moreover, the high concentration of natural fluorine and catechins in the tea plant provides a significant protective effect against dental caries and oral pathogens by inhibiting the bacteria responsible for plaque and bad breath **(Neha Sharma *et al.*2022).**

In modern therapeutic contexts, extensive research highlights the cardiovascular benefits of green tea, specifically its ability to inhibit coronary atherosclerosis by reducing low-

density lipoprotein (LDL) and triglycerides while elevating high-density lipoprotein (HDL) levels. Additionally, green tea plays a critical role in managing chronic conditions; it assists in glycemic regulation and may delay the progression of Type 1 diabetes. Its potent polyphenols (GTP) exhibit strong anticancer activities by inhibiting tumor cell invasion and inducing apoptosis. Neuroprotective effects are also evidenced by its ability to reduce the accumulation of beta-amyloid proteins in the brain a key factor in preventing Alzheimer's disease—while providing hepatoprotective benefits against various toxins like alcohol **(Neha Sharma *et al.* 2022).**

The pharmacological potential of green tea is further rooted in its superior antioxidant capacity. Biochemical assays, such as DPPH and TOSC, have demonstrated that green tea extracts can effectively scavenge free radicals even at very low concentrations (IC50). This activity is primarily attributed to its polyphenolic content, which follows the potency order: EGCG > ECG > EGC > EC. These compounds also stimulate the activity of endogenous antioxidant enzymes, including Superoxide Dismutase (SOD), Catalase, and Glutathione Peroxidase (GSH-Px), thereby protecting the brain and liver from oxidative stress-induced damage **(Tiantian Zhao *et al.*2022).**

Regarding metabolic applications, green tea catechins play a pivotal role in weight management and obesity prevention. Clinical and animal studies indicate that regular consumption can suppress food intake and reduce fat tissue accumulation. This anti-obesity effect is driven by thermogenesis (increased energy expenditure) and fat oxidation, resulting from a synergistic interaction between caffeine and EGCG. These constituents inhibit the enzyme catechol-O-methyltransferase (COMT), leading to prolonged levels of norepinephrine and enhanced lipid metabolism. Additionally, green tea theaflavins have shown a significant ability to inhibit enzymes involved in cholesterol biosynthesis, providing a dual benefit for metabolic health **(Raymond Cooper *et al.*2005).**

Finally, the potential applications of tea extend to mental health and antiviral defense. The amino acid L-theanine facilitates the generation of alpha waves in the brain, promoting a state of relaxation and mental focus while reducing cortisol levels during periods of stress. Moreover, green tea exhibits broad-spectrum antiviral properties; it inhibits the entry of viruses such as HIV and influenza into host cells and has shown potential as a dietary supplement in the prevention and treatment of COVID-19 by interacting with viral proteins. Recent dermatological research also suggests that topical applications of EGCG can restore

Glutathione (GSH) levels in the skin, providing a protective barrier against ultraviolet (UV) radiation damage **(Raymond Cooper *et al.*2005).**

Chapter II :
Bioactive Composition and Biological Activities.

II.1 Phytochemical Profile of Green Tea (*Camellia sinensis*):

The biochemical landscape of green tea is exceptionally diverse, containing over 500 chemical constituents isolated to date, including more than 400 organic and 40 inorganic compounds **(Mansoori R *et al*. 2022)**. As a non-fermented tea, it uniquely preserves its original chemical integrity.

II.1.1 The Polyphenolic Complex (20–30% of Dry Weight) :

Polyphenols represent the primary antioxidant fraction, encompassing approximately **30 types** of compounds:

II.1.1.1 Catechins (The Dominant Fraction): These constitute the core of tea polyphenols (30–42% of solids). The analytical concentration of total catechins reaches (Preeti Arya et al;2018). The main identified structures include:

1. **EGCG (Epigallocatechin gallate):** The most potent constituent (50% of total catechins). **(Preeti Arya *et al*.2018).**
2. **ECG (Epicatechin gallate):** Represents 20% of catechins **(Maria Eduardo et al.2007).**
3. **Other Catechins:** Catechin (C), Epicatechin (EC), and Epigallocatechin (EGC) .

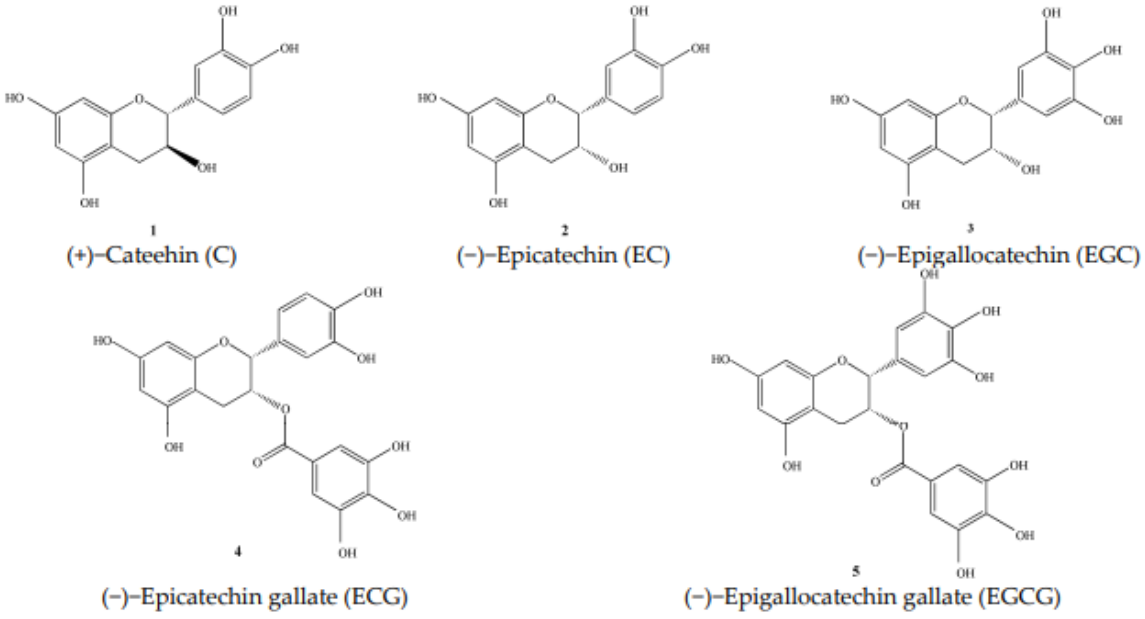
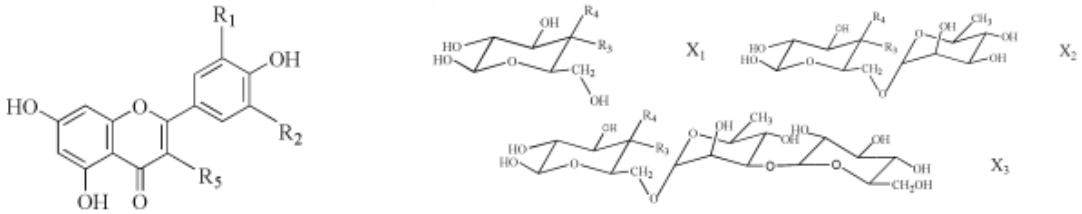


Figure 14: chemical structures of catechins isolated from green tea.

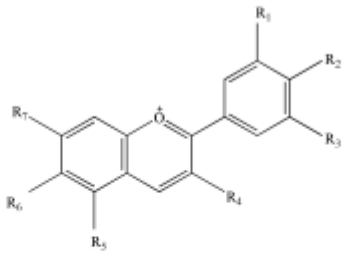
II.1.1.2 Flavonoids (Flavonol Glycosides): Green tea is rich in glycosides of Myricetin, Quercetin, and Behenyl. These are linked to carbohydrate chains (glucose, galactose, rhamnose) . As presented in Figure 15.

Anthocyanins: Water-soluble pigments (e.g., **Cyanidin, Delphinidin**). Despite their low concentration, they significantly impact the quality and bitter taste of the tea **(Mansoori R *et al*;2022)**. As presented in Figure 16.



6	Myricetin-3-rhamnosylglucoside	$R_1 = OH$	$R_2 = OH$	$R_3 = OH$	$R_4 = H$	$R_5 = X_2$
7	Myricetin-3-galactoside	$R_1 = OH$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = X_1$
8	Myricetin-3-glucoside	$R_1 = OH$	$R_2 = OH$	$R_3 = OH$	$R_4 = H$	$R_5 = X_1$
9	Quercetin-3-glucosyl-rhamnosyl-galactoside	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = X_3$
10	Quercetin-3-glucosyl-rhamnosyl-glucoside	$R_1 = H$	$R_2 = OH$	$R_3 = OH$	$R_4 = H$	$R_5 = X_3$
11	Quercetin-3-rhamnosylgalactoside	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = X_2$
12	Quercetin-3-rhamnosylglucoside	$R_1 = H$	$R_2 = OH$	$R_3 = OH$	$R_4 = H$	$R_5 = X_2$
13	Quercetin-3-galactoside	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = X_1$
14	Quercetin-3-glucoside	$R_1 = H$	$R_2 = OH$	$R_3 = OH$	$R_4 = H$	$R_5 = X_1$
15	Kaempferol-3-glucosyl-rhamnosyl-galactoside	$R_1 = H$	$R_1 = H$	$R_3 = H$	$R_4 = OH$	$R_5 = X_3$
16	Kaempferol-3-glucosyl-rhamnosyl-glucoside	$R_1 = H$	$R_1 = H$	$R_3 = OH$	$R_4 = H$	$R_5 = X_3$
17	Kaempferol-3-rhamnosylgalactoside	$R_1 = H$	$R_1 = H$	$R_3 = H$	$R_4 = OH$	$R_5 = X_2$
18	Kaempferol-3-rhamnosylglucoside	$R_1 = H$	$R_1 = H$	$R_3 = OH$	$R_4 = H$	$R_5 = X_2$
19	Kaempferol-3-galactoside	$R_1 = H$	$R_1 = H$	$R_3 = H$	$R_4 = OH$	$R_5 = X_1$
20	Kaempferol-3-glucoside	$R_1 = H$	$R_1 = H$	$R_3 = OH$	$R_4 = H$	$R_5 = X_1$

Figure 15 : Chemical structures of flavonoids isolated from green tea



21	Cyanidin	$R_1 = OH$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = OH$	$R_6 = H$	$R_7 = OH$
22	Delphindin	$R_1 = OH$	$R_2 = OH$	$R_3 = OH$	$R_4 = OH$	$R_5 = OH$	$R_6 = H$	$R_7 = OH$
23	Pelargonidin	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = OH$	$R_6 = H$	$R_7 = OH$
24	Malvidin	$R_1 = OCH_3$	$R_2 = OH$	$R_3 = OCH_3$	$R_4 = OH$	$R_5 = OH$	$R_6 = H$	$R_7 = OH$
25	Petunidin	$R_1 = OH$	$R_2 = OH$	$R_3 = OCH_3$	$R_4 = OH$	$R_5 = OH$	$R_6 = H$	$R_7 = OH$

Figure 16: chemical structures of anthocyanins isolated from green tea.

II.1.1.4 Condensed Tannins:

Formed by catechin polymerization, providing antimicrobial and protein-stabilizing properties **(Mansoori R *et al*.2022).**

III.1.1.5 Phenolic Acids:

Includes **Gallic acid**, along with Chlorogenic, Caffeic, and Ellagic acids **(Jing Tian *et al*;2021).**

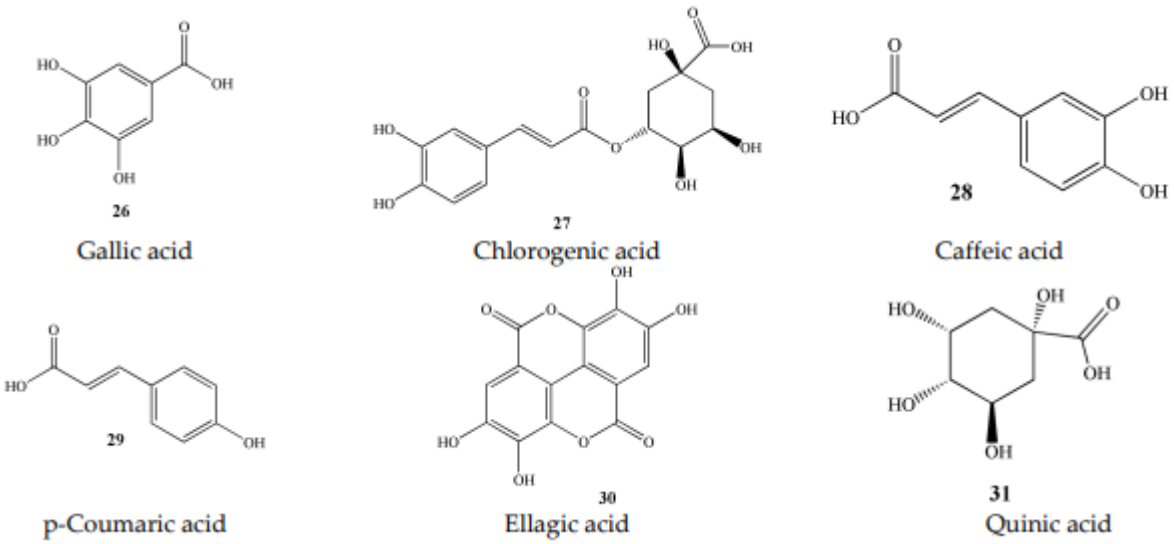


Figure 17: Chemical structures of phenolic acids isolated from green tea.

II.1.2 Alkaloids :

The stimulating effects of the extract are attributed to its purine alkaloids:

- **Caffeine:** The most abundant alkaloid, ranging from **2% to 5% (Preeti Arya *et al*.2018).**
- **Theophylline & Theobromine:** Present in smaller quantities, forming the base for the tea's physiological stimulant effect **(Guo-Yi Tang *et al.2019)***

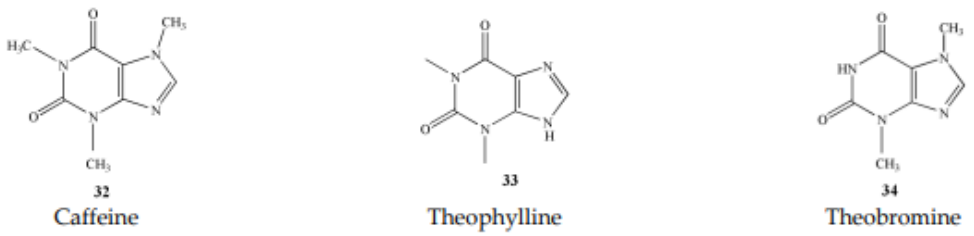


Figure 18 :Chemical structures of alkaloids isolated from green tea.

II.1.3 Amino Acids:

The profile and concentration of amino acids are among the most critical factors influencing the quality of green tea, which typically contains between 1% and 4% of these compounds on a dry weight basis. To date, 26 amino acids have been identified, including 20 proteinogenic and 6 non-proteinogenic types. Notably, L-Theanine is the most abundant, representing approximately 50% of the total amino acid content, and is renowned for its neuroprotective effects. Other significant acids include Glutamic acid, Arginine, Serine, and Aspartic acid, alongside the neuroprotector γ-aminobutyric acid (GABA), which is present in lower concentrations but holds substantial biological importance **(Zhao, T *et al.*2022).**

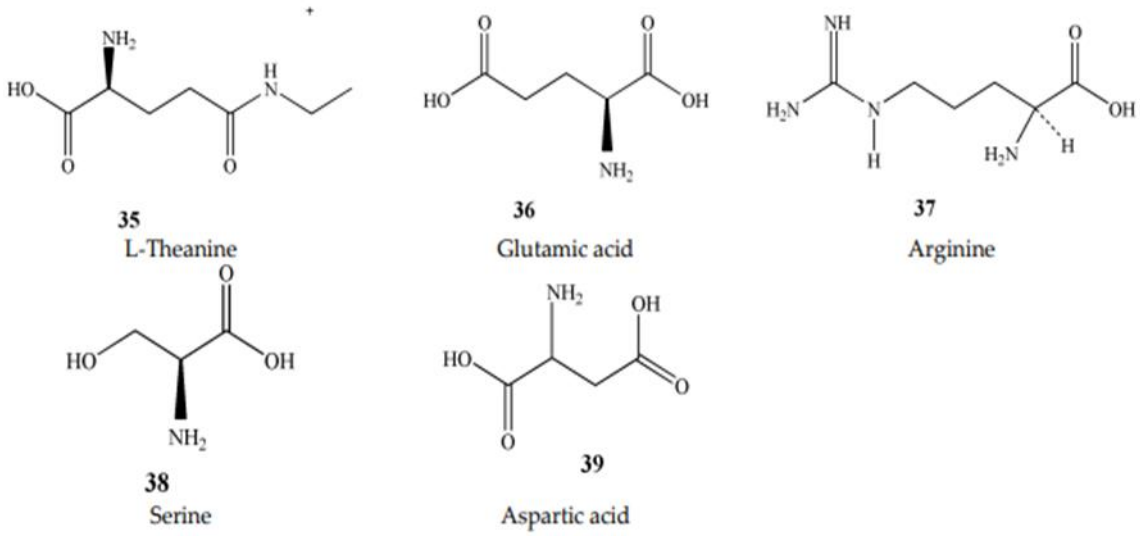


Figure 19: chemical structures of amino acids isolated from green tea.

II.1.4 Organic Acids, Carbohydrates, and Minerals :

- **Organic Acids:** Oxalic, Malic, Citric, and Ascorbic (Vitamin C) acids, which maintain the stability and pH of the extract **[Figure 20.]**.
- **Carbohydrates:** Monosaccharides (Glucose, Fructose) and Polysaccharides (Cellulose, Pectin) **(Zhao, T *et al.*2022).**
- **Minerals (Ash):** Significant levels of **Phosphorus (P)** and **Potassium (K)**, essential for enzymatic co-factors **(Zhao, T *et al.*2022).**.

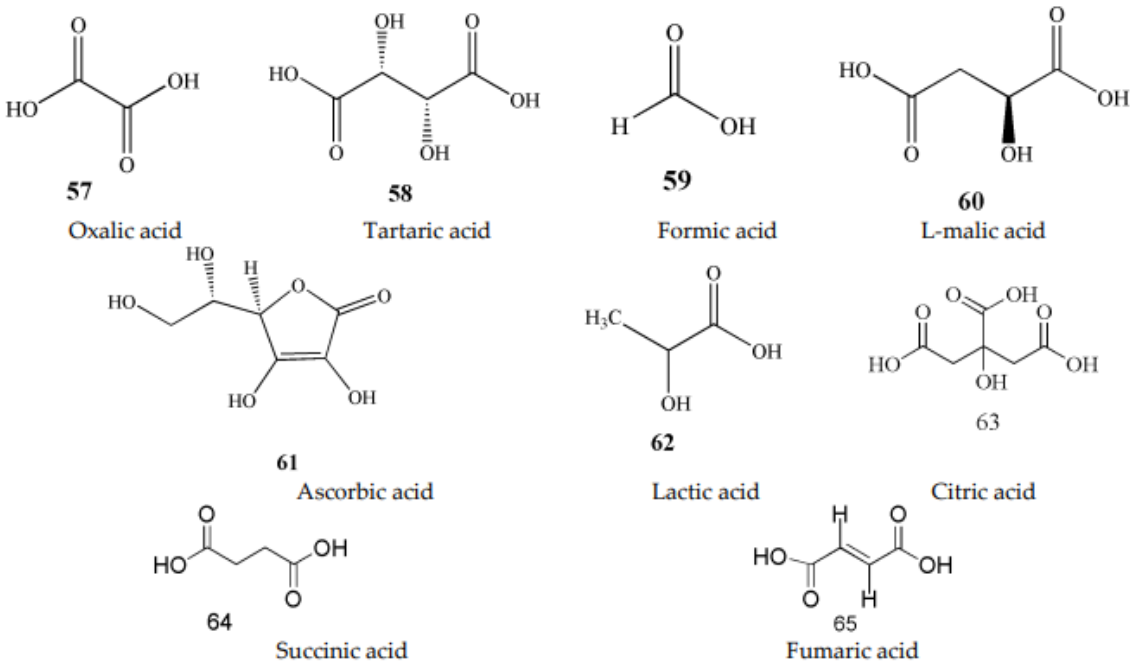


Figure 20: chemical structures of organic acids isolated from green tea.

II.1.5 Lipids and Sterols (1–2% dry weight) :

Plant sterols (β-sitosterol, stigmasterol) and minor fatty acids for membrane stability.

II.1.6 Additional Vitamins :

B vitamins (B1/thiamine, B2/riboflavin, B3/niacin), Vitamin E (tocopherols), Vitamin K (phylloquinone).

II.1.7 Pigments and Enzymes : Chlorophyll (green coloration), carotenoids (β-carotene, xanthophylls); enzymes (peroxidase, polyphenol oxidase) influenced by processing.

II.1.8 Volatile Aromatic Compounds (Aroma Profile):

The distinctive sensory identity and aroma of green tea are primarily attributed to a diverse array of volatile aromatic substances. Although these components represent a relatively minute fraction of the tea's chemical composition ranging from **0.005%** to **0.020%** of the total content their structural complexity is immense.

Extensive analytical research has been dedicated to characterizing these volatile profiles, leading to the continuous discovery and identification of new aromatic constituents.

These compounds, which include alcohols, aldehydes, ketones, and esters, work in synergy to define the "fresh" and "floral" notes characteristic of non-fermented tea. Despite their low concentration, they play a decisive role in the organoleptic evaluation and the overall commercial quality of the final product. **(Zhao, T *et al*.2022).**

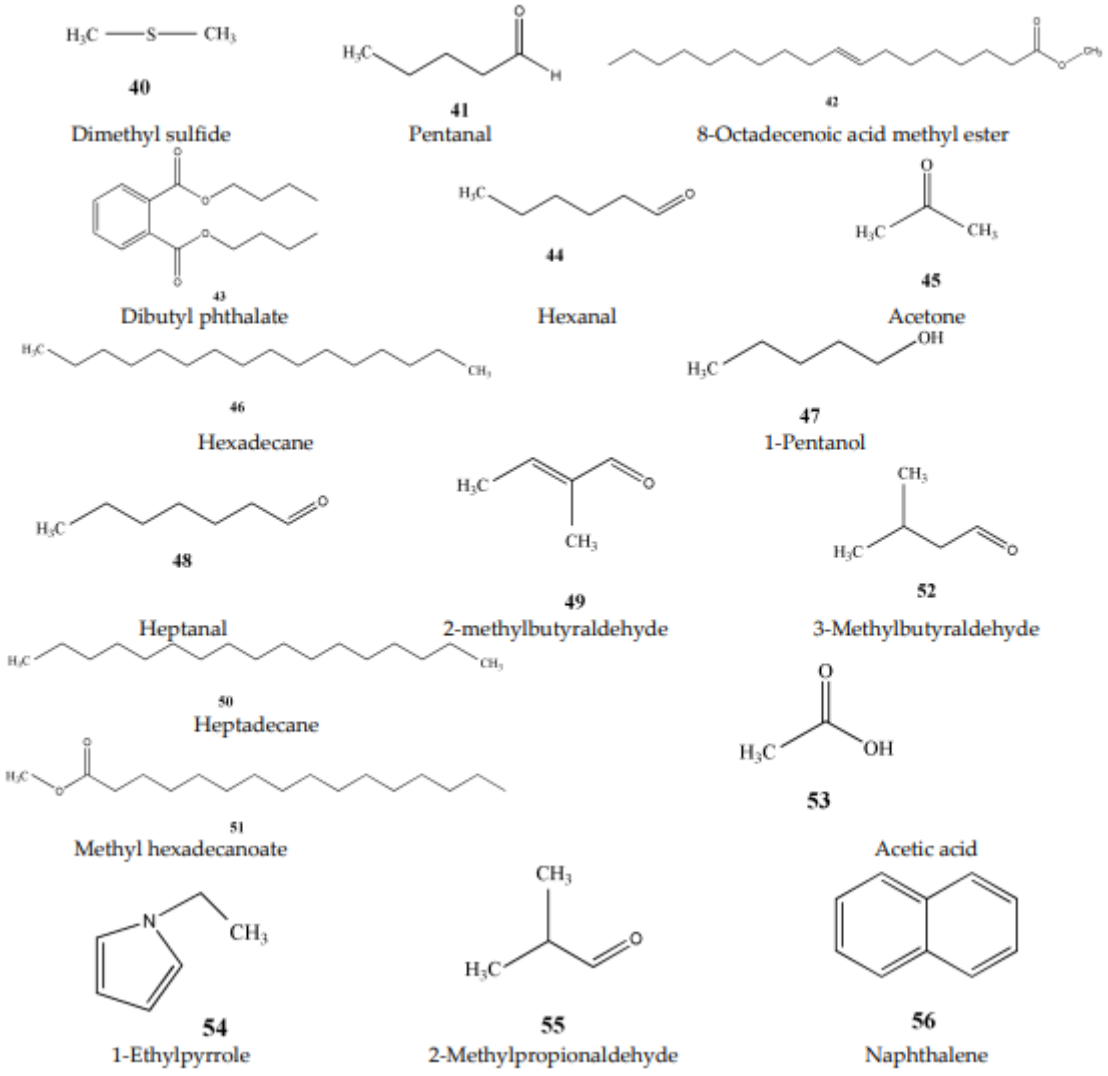


Figure 21: Chemical structures of aromatic ingredients isolated from green tea.

II.2 Biological Activities :

II.2.1 Antioxidant Activity :

II.2.1.1 Free Radicals: Concept and Sources:

Free radicals are defined as highly reactive and unstable chemical species whether atoms, molecules, or ions characterized by the presence of one or more unpaired electrons in their outer orbitals. This unstable structure grants free radicals a superior ability to attack surrounding molecules to abstract electrons, thereby causing molecular damage **(Gülçin, 2025).**

In biological systems, these radicals arise via two pathways:

Endogenous Sources: Produced as natural byproducts of cellular metabolism, particularly in the mitochondria during cellular respiration, or through xanthine oxidase activity, peroxisomal reactions, and phagocytosis during inflammatory responses.

Exogenous Sources: Such as exposure to environmental pollution, radiation, cigarette smoke, and certain pharmaceutical compounds **(Gülçin, 2025).**

II.2.1.2 Oxidative Stress :

While low and balanced levels of free radicals play vital roles in cellular signaling and immune defense, an imbalance favoring free radicals leads to Oxidative Stress. This condition attacks essential biomolecules such as proteins, lipids, carbohydrates, and nucleic acids (DNA and RNA). The oxidation of nucleosides within DNA is a prominent example of such damage, which is directly linked to the development of chronic diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders **(Gülçin, 2025).**

II.2.1.3 Antioxidants: Definition and Chemical Mechanisms:

Antioxidants are compounds capable of delaying or preventing the oxidation of a substrate, even when present at very low concentrations. These compounds operate through three primary chemical mechanisms **(Santos *et al.* 2018):**

1. **Hydrogen Atom Transfer (HAT):** Where the antioxidant donates a complete hydrogen atom to the free radical to stabilize it.

2. **Single Electron Transfer (SET):** Neutralizing the oxidative threat by donating a single electron to the free radical.

3. **Metal Chelation**: Binding to transition metals (such as iron and copper) to prevent them from catalyzing reactions that produce free radicals.

II.2.1.4 Classification of Antioxidant Defense Systems:

Enzymatic Antioxidants (Enzymatic System):

These represent the primary and secondary lines of defense in the body, working synergistically to reduce levels of reactive oxygen species (ROS) and protect cellular membranes **(Nimse *et al*. 2014).**

Non-Enzymatic Antioxidants and Natural Compounds

There is an increasing interest in natural plant sources as alternatives to synthetic substances. Key components include:

1. Vitamins:

- Vitamin E: Acts as a chain-breaker in lipid membranes.
- Vitamin C: A direct free radical scavenger that also regenerates consumed Vitamin E.
- Vitamin A: Plays a vital role in protecting low-density lipoproteins (LDL) from metal-induced oxidation.

2. Phenolic Compounds and Bioflavonoids:

Their effectiveness depends on their aromatic structure and the number and position of hydroxyl groups (-OH); substitutions at the "ortho" and "para" positions enhance their radical-scavenging capacity.

- Anthocyanidins: Their strength stems from the "ortho-dihydroxy" structure and the conjugated double bond, enabling efficient radical scavenging and metal chelation **(Nimse et al., 2014).**

- Quercetin Caution: Acts as a DNA protector at low copper concentrations but may become a pro-oxidant at high metal concentrations **(Nimse et al., 2014).**

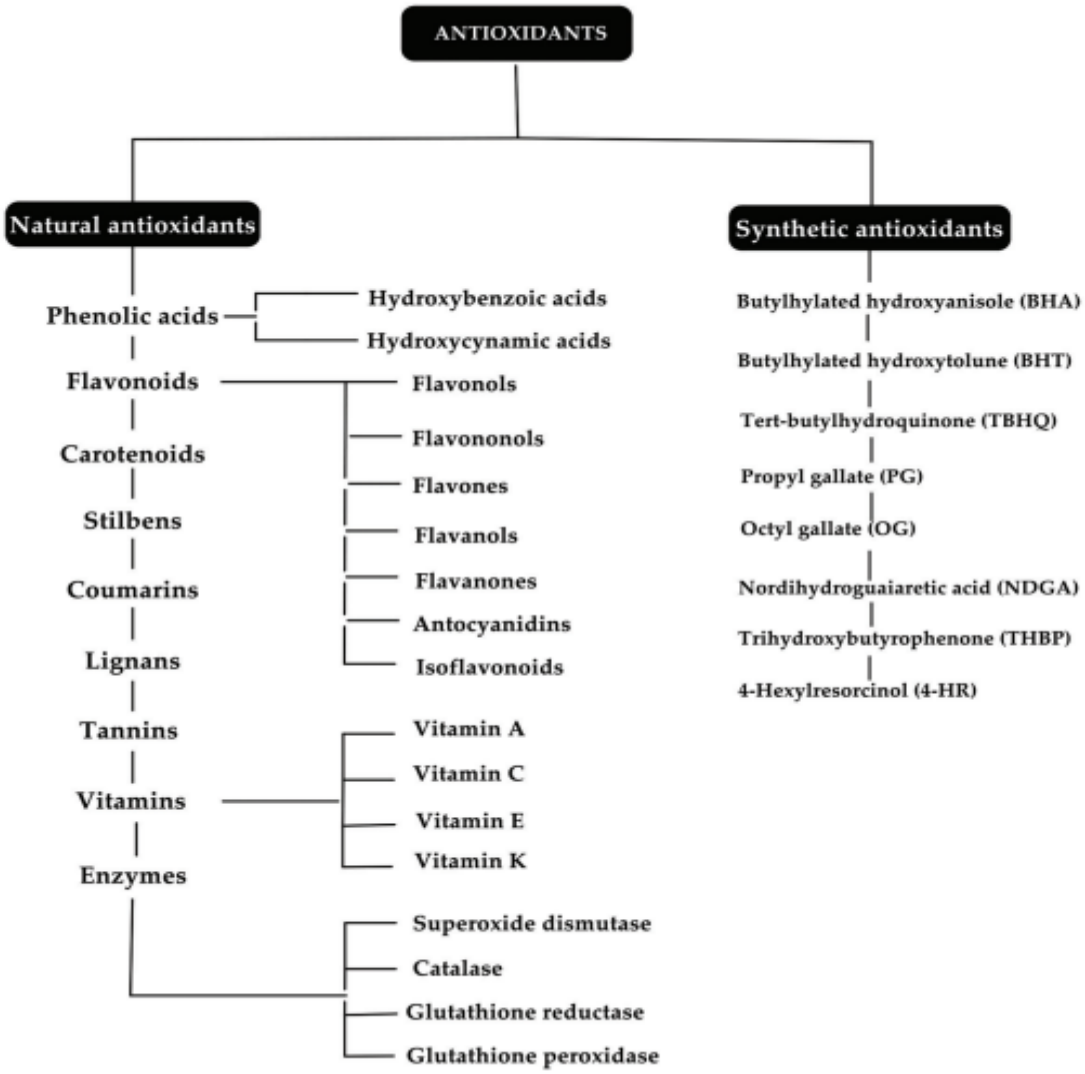


Figure 22: Classification of antioxidant compounds **(Adapted from Gülçin 2025**).

II.2.1.5 Evaluation of Antioxidant Activity:

In recent years, there has been a significant increase in the search for and identification of safe, natural antioxidants, particularly those of plant origin **(Jian-Ming Lü *et al*.2010).**

Consequently, the *in vitro* evaluation of these antioxidants has become an indispensable and essential step. These methods are based on physicochemical principles that allow for the quantification of a compound's ability to scavenge free radicals or reduce oxidizing agents. Among the most widely utilized assays are non-competitive assays such as DPPH, FRAP, and ABTS, measured spectrophotometrically. In these assays, there is no competing target molecule, as the antioxidant reacts directly with the chromogenic reagent **(Prior et al., 2005).**

While FRAP relies strictly on Single Electron Transfer (SET), both DPPH and ABTS are considered mixed-mode assays. They function through a combination of Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET), where the dominant mechanism is often influenced by the solvent's nature and the pH of the medium. These tests are highly favored due to their simplicity, rapidity, and cost-effectiveness, making them efficient tools for the rapid screening of antioxidant activity in various natural extracts **(Prior *et al*. 2005; Flieger *et al*. 2021).**

Table 2: Methods most commonly used to evaluate antioxidant activity in vitro (Adapted from **(Norma Francenia Santos *et al.*2018).**

Method	Reaction mechanism	Characteristics
Inhibition of 2,2-diphenyl-1-picrylhydracyl radical (DPPH•)	SET or HAT	Colorimetric method based on the measurement of the scavenging capacity of antioxidants towards DPPH•
Inhibition of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonicacid) (ABTS•+) cation radical	SET or HAT	Colorimetric method to evaluate the decay of ABTS•+ in the presence of an antioxidant agent
Ferric-reducing antioxidant power (FRAP)	SET	Colorimetric method that evaluates the reduction of Fe3+-tripyridyl triazine complex (Fe3+-TPTZ) by turning it into a ferrous form (Fe2+-TPTZ)

II.2.2 Anti-inflammatory Activity:

Inflammation is the body's first and most important line of defense against tissue damage. In this process, immune responses and cellular changes work together to eliminate pathogens and repair injured areas. However, if the body fails to control this reaction, it can persist and turn into a chronic condition **(Xiujing Huang *et al*.2024)**. Many factors can trigger inflammation, including mechanical injuries, microbial infections, burns, autoimmune disorders, or toxic chemicals. In all these cases, the body works to neutralize the external threat to allow for healing and to return the tissues to their stable, normal state (Homeostasis) **(Sriaandhal Sabalingam *et al* .2025).**

This defensive system is managed by chemical mediators released by damaged tissues and migrating immune cells. Clinically, this results in the five well-known signs of inflammation: pain, swelling, redness, heat, and loss of function in the affected area **(Sriaandhal Sabalingam *et al*.2025).**There are two different time-based pathways for this response: the Acute Pathway, which aims to contain the injury immediately and restore tissue balance, and the Chronic Pathway, which occurs when the body fails to end the initial inflammatory response. This chronic state is a major factor in the development of many complex diseases.

II.2.2.1 The Molecular Mediators and Pathways of Inflammation :

The inflammatory process is managed through a complex network of cellular mediators, initiated by signal sensors and transcription factors such as NF-κB and AP-1, which serve as the primary drivers of inflammation . Due to the technical difficulties in measuring these factors directly within the cells , researchers focus on quantifying the resulting "biomarkers," most notably enzymes and cytokines **(DSHS Peiris *et al.*2024).**

In this context, Arachidonic Acid (AA) plays a pivotal role as the primary substrate released from damaged cell membranes to initiate inflammatory pathways. This acid is distributed between two main enzymatic pathways:

- The COX-2 Pathway: Which consumes arachidonic acid to synthesize prostaglandins, the mediators responsible for pain and fever **(Xiujing Huang *et al*.2024).**
- The 5-LOX Pathway: Which utilizes the same substrate to produce leukotrienes (LTs) **(Xiujing Huang *et al*.2024)**, which enhance inflammatory intensity and allergic reactions **(Wang *et al*. 2021)**.

Additionally, cytokines (such as TNF-α and ILs) and nitric oxide contribute to reinforcing this inflammatory environment and guiding cell differentiation. Although current pharmaceuticals aim to inhibit these pathways to reduce symptoms, their significant side effects necessitate the search for natural therapeutic alternatives that are safer and more biocompatible with the human body **(Sriaandhal Sabalingam *et al*.2025).**

II.2.2.2 In vitro assays for evaluating anti-inflammatory activity :

Plants serve as a rich source of bioactive compounds known as phytochemicals, such as terpenoids, alkaloids, and flavonoids. The significance of these compounds lies in their potent ability to suppress inflammation (Anti-inflammatory activity) by modulating signaling pathways and inhibiting pro-inflammatory mediators (**Sriaandhal Sabalingaet al; 2025).**

In the early stages of scientific research and drug development, in vitro studies are considered a pivotal investigative tool. They provide a controlled environment that allows researchers to isolate specific variables accurately, away from the complex interactions within living organisms, while also being a cost-effective alternative to in vivo studies (**Sriaandhal Sabalingaet al; 2025)** .

This work focuses on a widely used in vitro assay to evaluate anti-inflammatory activity, which is:

- Stabilization of human red blood cell (HRBC) membrane test.

Stabilization of human red blood cell (HRBC) membrane test .

The membrane stabilization method evaluates the capacity of compounds to protect biological membranes against lysis induced by osmotic or thermal stress, utilizing erythrocytes as an alternative laboratory model for lysosomal membranes due to their structural similarities **(Anosike CA *et al*. 2012) (Aidoo DB *et al*.2021).**

The significance of this mechanism lies in the fact that stabilizing the lysosomal membrane prevents the leakage of hydrolytic enzymes responsible for tissue destruction and exacerbated inflammation; thus, inhibiting hemolysis serves as a direct indicator of anti-inflammatory efficacy. This assay is distinguished as a simple, cost-effective preliminary screening tool and an ethical alternative to animal models, despite its limitations in fully replicating the complexities of the in vivo environment **(Fujiati F, *et al*.2022)** .

II.2.3 Antimicrobial activity:

Bacterial infections represent a growing global health challenge that affects millions annually **(Farouk Boudou *et al*.2025).**

Antimicrobial resistance (AMR) has become a daunting concern for the scientific community due to its severe implications, as infectious diseases have become increasingly difficult to treat. With the rise of multi-drug resistant (MDR) bacteria and the limited development of new antibiotic classes, researchers have shifted their focus toward exploring natural and synthetic compounds as potential therapeutic alternatives. In this context, in vitro assays play a pivotal role in the discovery and development of these agents, providing fundamental insights into their efficacy and molecular mechanisms of action **(Tanim Jabid Hossain.2024)**.

Among the most promising sources under development are medicinal plants, which possess a diverse arsenal of bioactive phytochemicals that serve as a natural defense system. Key groups within these compounds include polyphenols and terpenoids. Among these botanical sources **(Tanim Jabid Hossain.2024),** tea *(Camellia sinensis*) stands out due to its exceptional richness in phenolic compounds and its well-documented health benefits, particularly its potent antimicrobial activity.

Phenolic compounds identified in this plant, such as epigallocatechin gallate (EGCG) and quercetin, exhibit significant inhibitory activity against resistant strains. For instance, EGCG enhances the synergistic efficacy of β-lactam antibiotics against MRSA by targeting and disrupting the bacterial cell wall. Meanwhile, quercetin plays a crucial role in inhibiting biofilm formation, which is a decisive factor in antibiotic resistance **(Farouk Boudou *et al*.2025)**.

II.2.3.1 Antimicrobial Activity Evaluation :

To evaluate the antimicrobial activity of both establish ed and novel compounds under investigation, a variety of techniques are employed. Among these methods, the agar diffusion assay was selected due to its simplicity, cost-effectiveness, and rapid generation of results, which serve as a fundamental basis for advanced research and more complex methodology.

II.2.3.1.1 Agar diffusion based assay:

The agar diffusion technique is based on the migration of active compounds from their source into the culture medium to inhibit microbial growth, with the zone of inhibition diameter serving as the primary criterion for determining the relative potency of the tested substance .

This method is characterized as a cost-effective and flexible screening tool, providing precise qualitative data on the efficacy of plant extracts prior to more complex assays, making it a fundamental pillar in antimicrobial research **(Tanim Jabid Hossain;2024).**

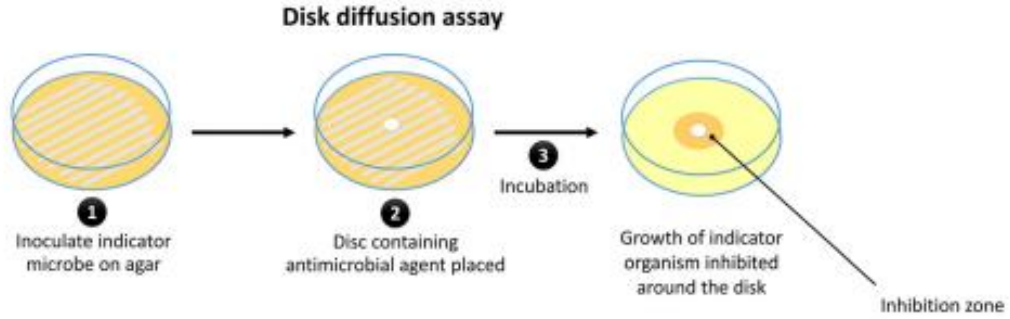


Figure 23: Schematic representation of the disk diffusion **(Adapted from Tanim Jabid Hossain;2024).**

Part Two: Experimental Part.

Chapter III:
Materials and *Methods*

III. Materials and Methods

III.1 Introduction:

This study focuses on the phytochemical and biological evaluation and comparison of two commercial tea brands available in the local market. Our methodology involved the preparation of three distinct extracts: ethanolic, infusion, and decoction.

The main objectives of our study are focused on the following key axes :

- To conduct a phytochemical screening in order to identify the different chemical families present in our tea extracts (phenolic compounds, flavonoids, etc.).
- To evaluate the antioxidant capacity of the extracts using two complementary methods: the DPPH radical scavenging assay and the FRAP (Ferric Reducing Antioxidant Power) assay.
- To assess the anti-inflammatory activity through an *in vitro* assay using human red blood cell (HRBC) membrane stabilization, to determine the therapeutic potential of the extracts.
- To evaluate the antimicrobial activity against various bacterial strains and Fungal strains and using sensitivity tests, in order to determine the inhibitory power of our extracts against these microorganisms.

All experimental procedures were conducted at Dr. Moulay Tahar University of Saida, within the Faculty of Natural and Life Sciences. Specifically, the work was carried out in the Biochemistry Laboratory (Lab No. 10) and the Microbiology Laboratory (Lab No. 11) of the Biology Department.

III.2 Plant Materials

The plant material used in this study consists of two commercial brands of green tea ***(Camellia sinensis)*** "El-Berrad" and "El-Rakim". These brands were selected due to their high consumption rates among the local population in Saida (Algeria).

The samples, consisting of dried tea leaves, underwent a standardized preparation process: they were finely ground using an electric grinder and then sieved to obtain a fine, homogeneous powder. This powder was stored in airtight containers in a dark, dry place until further use for extraction.

(a) (b)

Figure 24 :Commercial samples of green tea used in this study a: El-Berrad b: El-Rakim.

III.3 preparation of Extracts

III.3.1 Aqueous Infusion

The aqueous infusion was prepared by heating 100 mL of distilled water in a beaker on a hot plate until boiling. Once boiling, the beaker was removed from the heat source, and 5 g of the plant powder was added to the water. The mixture was stirred and allowed to steep while cooling to room temperature. Finally, the mixture was filtered, and the resulting filtrate was collected for subsequent analysis. (**KADDOURI Mebarka .2024).**

III.3.2 Aqueous Decoction (Reflux Extraction):

The decoction was carried out using a reflux setup to prevent solvent loss. 5 g of the plant powder was mixed with 100 mL of distilled water in a round-bottom flask. The flask was placed in a **heating mantle** (Chauffe-ballon) and connected to a reflux condenser. The mixture was heated to a stable boiling point and maintained for one hour. After cooling, the solution was filtered, and the aqueous filtrate was recovered. by **(KADDOURI Mebarka .2024).**

III.3.3 Ethanolic Decoction (Ethanolic Extraction):

The same procedure as the aqueous decoction was followed, replacing the distilled water with ethanol. 5 g of the plant powder was mixed with 100 mL of ethanol in a round-bottom flask. The mixture was heated under reflux using a **heating mantle** for one hour to ensure exhaustive extraction while preventing ethanol evaporation. Upon cooling, the mixture was filtered to obtain the alcoholic filtrate. **(KADDOURI Mebarka .2024).**

At the end of the extraction process, the extracts were concentrated under vacuum using a rotary evaporator (BÜCHI) at temperatures of 37°C and 45°C, respectively. Following concentration, the resulting extracts were transferred into sterile watch glasses and placed in an oven at 37°C until complete dryness.

The resulting dry extracts were stored in sterile, dry, and airtight amber glass bottles. To prevent the degradation of phenolic compounds and other bioactive constituents, the samples were kept in a dark environment inside a refrigerator at 4°C until further use. This procedure was strictly followed to avoid photo-oxidation and maintain the stability of the plant metabolites.

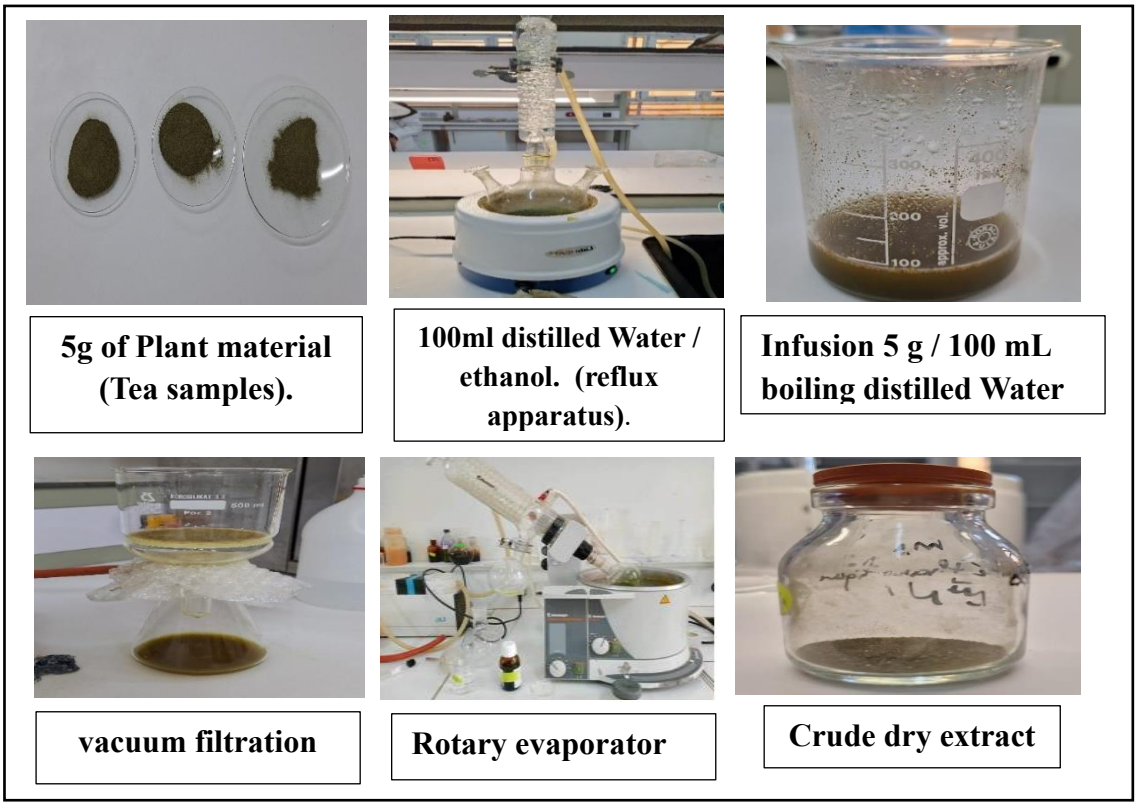


Figure 25 :Preparation of three extracts (Infusion, Aqueous and Ethanolic decoctions).

III.3.4 Extraction Yield

"The extraction yield refers to the mass of the obtained extract, expressed as a percentage relative to the initial mass of the plant material used for extraction. For each sample, the analysis was performed in duplicate. The plant yields were determined as dry extract using the following formula**:" (Ranebaye D. et al.2023).**

R (%) = (M1 - M2 / M3) *100

- M 1 : Weight of the watch glass containing the dry extract.
- M 2 : Weight of the empty watch glass.
- M 3 : Weight of the initial plant material (5g).

III.4 Phytochemical Screening :

In order to evaluate the quality and efficiency of the natural components found in commercial tea, and to explore their therapeutic potential, a phytochemical screening was conducted. This screening allows for the identification and classification of various bioactive chemical groups, including: tannins, flavonoids, anthocyanins, coumarins, reducing compounds, sterols, steroids, starch, alkaloids, and saponins. These phytochemical tests are based on solubility properties, specific coloration and precipitation reactions, as well as examinations under ultraviolet (UV) light.

Flavonoids :

A volume of 5 ml of the ethanolic extract was mixed with 1 ml of concentrated hydrochloric acid (HCl) and 1 g of magnesium turnings. The presence of flavonoids was confirmed by the appearance of a red or pink coloration. The results were recorded as follows: (+) for the presence of flavonoids and (-) for their absence **(Karumi et al., 2004).**

Tannins :

A volume of 1 ml of the ethanolic extract was mixed with 2 to 3 drops of a 1% ferric chloride (FeCl3) solution. After a few minutes of incubation, the appearance of a greenish coloration indicates the presence of catechol tannins, while a blue-black coloration reveals the presence of gallic tannins **(Karumi et al., 2004).**

Coumarins Test :

A quantity of 1 g of plant powder was dissolved in a few drops of hot water. The resulting solution was covered with filter paper moistened with diluted sodium hydroxide (NaOH) and then brought to a boil. The paper was subsequently examined under ultraviolet (UV) light. The appearance of intense fluorescence indicates the presence of coumarins **(Benmehdi, 2000).**

Reducing Compounds

To 1mL of the aqueous-ethanolic extract 20 drops of Fehling's solution were added, and the mixture was subsequently heated. The development of a brick-red precipitate serves as an indicator of a positive reaction **(Trease and Evans, 1987).**

Saponins

A 2mL volume of the aqueous infusion was combined with an equal volume of distilled water and shaken vigorously for 2min. The persistence of a stable foam layer after 15min confirms the presence of saponins **(Karumi et al., 2004).**

Starch

An aliquot of 5mL of the aqueous extract was treated with 10mL of NaOH followed by the starch reagent. The emergence of a blue-violet coloration signifies a positive test for starch presence **(Benmehdi, 2000).**

Anthocyanins Test:

A volume of 2 ml of the aqueous infusion was mixed with 2 ml of 2N hydrochloric acid (HCl). The appearance of a pink-red coloration, which turns blue-violet upon the addition of ammonia, indicates the presence of anthocyanins **(Benzeggouta, 2015).**

Alkaloids Test

This protocol was determined according to the method described by **(Paris *et al.* 1969)**, as cited by **KADDOURI Mebarka (2024).**

A maceration was performed for 24 hours using 2 grams of plant powder mixed with 50 ml of half-diluted sulfuric acid ($H_2 SO_4$) and distilled water. The mixture was

filtered and rinsed with water to obtain a final volume of 50 ml of filtrate. Subsequently, 1 ml of the macerate was introduced into two separate test tubes. To the first tube, 5 drops of Mayer's reagent were added, while 5 drops of Wagner's reagent were added to the second tube. The appearance of turbidity or a precipitate after 15 minutes indicates the presence of alkaloids.

Sterols and Triterpenes:

Two assays were performed to identify these compounds:

- **Assay 1**: Test for Sterols and Steroids:

A volume of 10 ml of the ethanolic extract was placed in an Erlenmeyer flask. After evaporation to dryness, the residue was dissolved in 10 ml of anhydrous chloroform. Then, 5 ml of the chloroform solution was mixed with 5 ml of acetic anhydride, followed by the addition of a few drops of concentrated sulfuric acid (H_2SO_4). The mixture was shaken and allowed to stand. A positive test is indicated by the appearance of a fleeting violet coloration that turns green (reaching maximum intensity within 30 minutes at 21°C).

- **Assay 2**: Test for Steroidal and Triterpenic Glycosides (Liebermann-Burchard Reaction):

This protocol was determined according to the method described by (**Trease et Evans, 1987),** as cited by **KADDOURI Mebarka (2024).**

This test involves evaporating 10 ml of the ethanolic extract to dryness. The resulting residue was dissolved in a mixture of acetic anhydride and chloroform (5:5, v/v). The mixture was then filtered, and the filtrate was treated with a few drops of concentrated sulfuric acid. The appearance of a blue-green coloration indicates the presence of steroidal glycosides, while a violet-green coloration reveals the presence of triterpenic glycosides **.**

III.5 Evaluation of Antioxidant Activity:

There are many advanced methods to evaluate the antioxidant activity of plant extracts. In this work, the DPPH Free Radical Scavenging Assay (2,2-diphenyl-1-

picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays were used to determine the antioxidant potential of our extracts.

III.5.1 DPPH Free Radical Scavenging Assay:

III.5.1.1 Principle of the assay:

The DPPH molecule contains stable free radicals, which give the solution its deep violet color. Upon the addition of an extract with antioxidant activity, it donates hydrogen atoms to these free radicals. As soon as the radicals capture the electrons, the violet color changes to pale yellow.

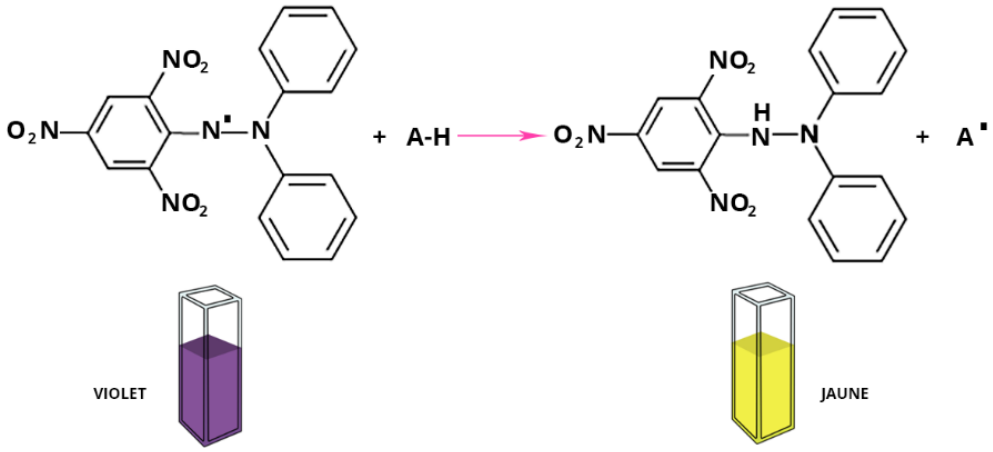


Figure 26 :Mechanism of DPPH radical scavenging **(Agro Paris Tech, (n.d)).**

The antioxidant test was carried out with the DPPH method. 50µl of each methanolic solution of the extracts at different concentrations (from 0.0125 to 5mg/ml) are added to 1.95 ml of the methanolic solution of DPPH (0.025g/l). At the same time, a negative control is prepared by mixing 50µl of methanol with 1.95 ml of the methanolic DPPH solution. The reading of the absorbance is made against a blank prepared for each concentration at 517nm after 30 min of incubation in the dark and at room temperature. The positive control is represented by a solution of a standard antioxidant; ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration the test is repeated 3 times. The results were expressed in percentage of inhibition (I%). **(Bougandoura & Bendimerad, 2012)**.

The Formula: $$I\%=[(Abs\ control-Abs\ test)/Abs\ control]\times 100$$

The IC50 values were determined graphically by linear regression.

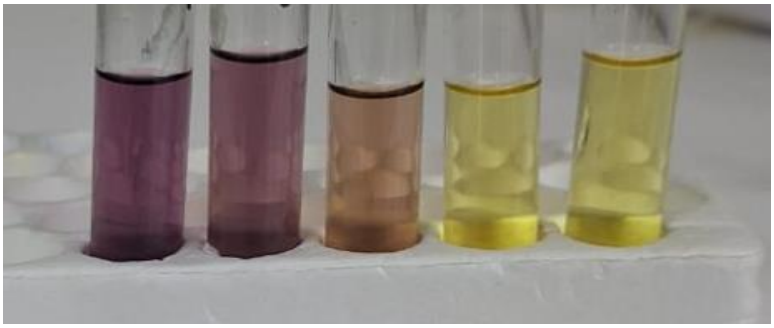


Figure 27 :Reduction of the DPPH● radical (purple color) to diphenyl-picrylhydrazine (yellow color).

III.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay :

III.5.2.1 Principle of the assay:

The FRAP (Ferric Reducing Antioxidant Power) assay measures the antioxidant capacity based on the ability of compounds to act as electron donors. In this reaction, the antioxidant provides an electron to the ferric-TPTZ (Fe^{3+}-TPTZ) complex, reducing it to the ferrous (Fe^{2+}) form. This chemical reduction results in a significant color transition, changing from a pale yellow to a dark green (or intense blue) hue, which is then measured spectrophotometrically **(Nwachukwu *et al*. 2021).**

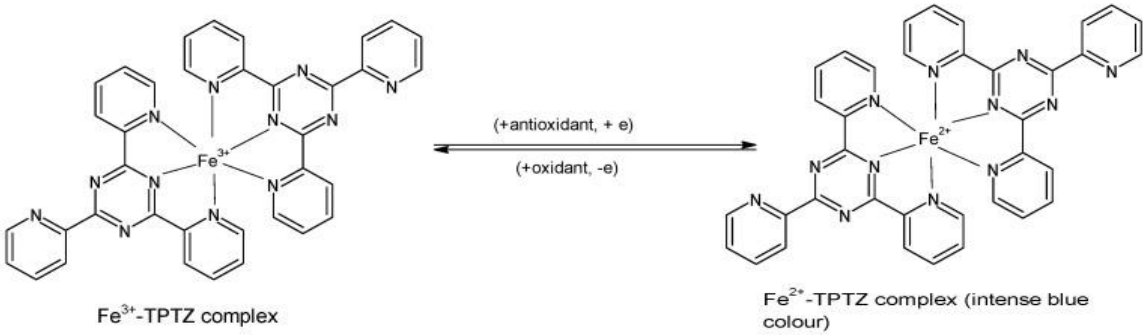


Figure 28: The reaction principle of the FRAP assay **(Nwachukwu *et al.* 2021).**

III.5.2.2 Experimental Procedure:

The reducing power of iron (Fe^{3+}) in the extracts was determined according to the method described by Oyaizu**.**

One milliliter of the extract at various concentrations was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% potassium ferricyanide (K_3 Fe $(CN)_6$) solution. The mixture was incubated in a water bath at 50°C for 20 minutes. Subsequently, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction, and the tubes were centrifuged at 3000 rpm for 10 minutes.

An aliquot (2.5 mL) of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous ferric chloride (Fe Cl_3) solution. The absorbance of the reaction mixture was measured at 700 nm against a similarly prepared blank, in which the extract was replaced with distilled water to calibrate the spectrophotometer (UV-VIS).

Ascorbic acid served as the positive control (standard antioxidant), with its absorbance measured under the same conditions as the samples. An increase in absorbance indicates an increase in the reducing power of the tested extracts. **(Bougandoura & Bendimerad, 2012, p. 16)**.

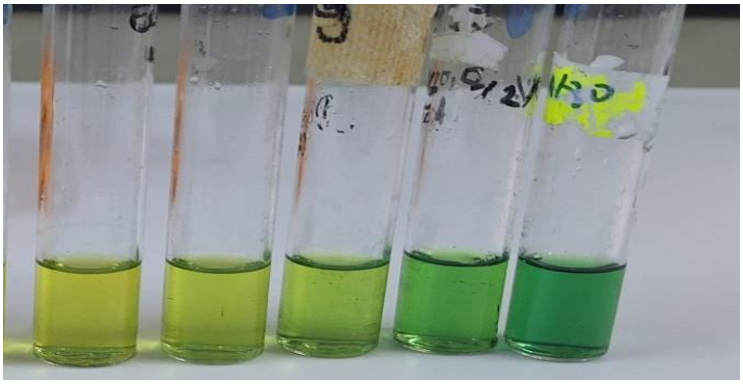


Figure 29: Reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}).

III.6 The anti-inflammatory activity test:

III.6.1 Red Blood Cell (Erythrocyte) Membrane Stabilization Test:

Within the framework of investigating the anti-inflammatory activity of the plant extract, the Red Blood Cell (RBC) membrane stabilization test was selected. This choice is justified by its operational simplicity and short duration. Furthermore, the results are visually detectable and can be precisely quantified using a spectrophotometer.

III.6.1.1 Principle of the assay :

Anti-inflammatory drugs function by stabilizing **lysosomal membranes**. Since the membranes of red blood cells (erythrocytes) are structurally similar to lysosomal membranes, they are used as an alternative to evaluate the ability of plant extracts and drugs to stabilize membranes exposed to lysis under hypotonic conditions. **(Gorla *et al.* 2018), (Nurtamin *et al.* 2018).**

III.6.1.2 Experimental Procedure :

The anti-inflammatory activity was evaluated using the Red Blood Cell (RBC) membrane stabilization assay. Fresh human whole blood was collected from a healthy volunteer who had not consumed any anti-inflammatory drugs for at least two weeks prior to the study. To ensure cell preservation, the blood was immediately mixed with an equal volume of Alsever's solution . The mixture was then centrifuged at 3000 rpm for 10 minutes, after which the supernatant was removed and the cell pellet was washed three times with an isotonic saline solution (0.9% NaCl). Subsequently, a 10% (v/v) erythrocyte suspension was prepared using the same isotonic saline.

Composition of the reaction mixture (total volume = 4.5 mL):

Table 3: Composition of the reaction mixture.

Components.	**Volume**
Phosphate buffer	1 mL
Hyposaline solution	2 mL
Erythrocyte suspension 10%	0,5 mL
Extract	1 mL

For the stabilization test, the reaction mixtures containing the plant extracts were incubated at 37 °C for 30 minutes, followed by another centrifugation step at 3000 rpm for 10 minutes to separate the cells. Finally, the absorbance of the resulting supernatant was measured at 560 nm using a UV-Visible spectrophotometer to quantify the degree of hemolysis and determine the membrane-stabilizing capacity of the extracts.

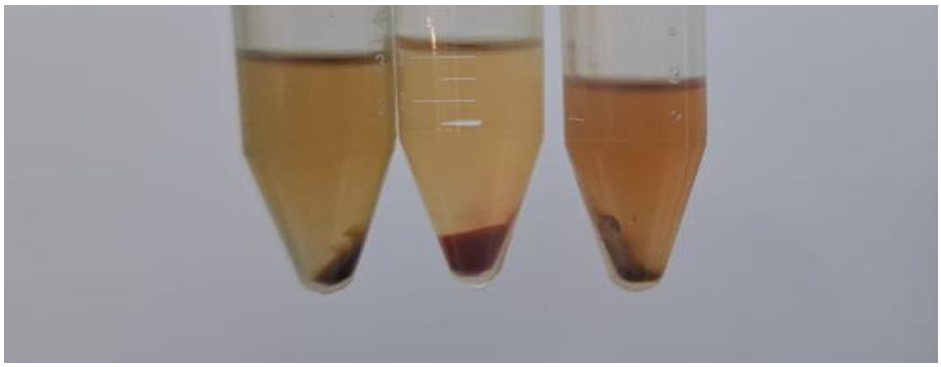


Figure 30: In vitro anti-inflammatory activity assay using the Human Red Blood Cell (HRBC) membrane stabilization method.

III.7 Antibacterial Activity Assay :

To evaluate the antimicrobial activity of the plant extracts *(Camellia sinensis)*, the agar diffusion method was selected.

III.7.1 Experimental Procedure :

The Antibacterial Activity Assay was evaluated according to the method described by **(Boudou *et al.*2025)**.

The bacterial strains listed in the table below were first cultured on nutrient agar and incubated at 37 °C for 24 hours. To prepare the inoculum, the bacterial colonies were suspended in a sterile saline solution and adjusted to a concentration of approximately 10^8 CFU/mL, standardized against the 0.5 McFarland turbidity scale.

Table 4: Sources of the strains used for the antimicrobial activity study.

Microorganisms	Code	Gram stain	Source
Escherichia coli.	ATCC 8739	Gram-	**MNHN**
Bacillus cereus.	ATCC 25921	Gram+	**LAPRONA**
Staphylococcus aureus.	ATCC 6538	Gram+	**LAPRONA**
Bacillus subtilis.	ATCC 6633	Gram+	**MNHN**
Aspergillus Niger	/	/	**Isolated from green tea.**
Penicillium zacinthae	/	/	**Isolated from green tea**

For the assay, 20 mL of sterile Mueller-Hinton agar (MHA) was poured into Petri dishes. Once the medium solidified, the agar surface was inoculated with the bacterial suspension using a sterile swab to ensure uniform distribution.

The plant extracts were dissolved in DMSO 1% to prepare various concentrations. Wells (6 mm in diameter) were aseptically punched into the inoculated agar medium, and each well was subsequently filled with 50 µL of the respective extract concentration.. For comparison, pure 1% DMSO was introduced into separate wells to serve as a negative control. while wells containing reference antibiotics served as the positive control.

Prior to incubation, the plates were placed in a refrigerator for 30 minutes to 1 hour to allow for the pre-diffusion of the extracts into the agar. Subsequently, The plates were incubated at 37°C for 24 hours for bacteria, and at 28°C for 7 days for fungi. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zones (in millimeters). A larger diameter indicates a stronger potency of the extract.

III.7.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) :

The broth microdilution method using sterile 96-well microplates was employed to determine the MIC and MBC values. Initially, 50 µL of sterile Mueller-Hinton broth (MHB) was distributed into each well. Subsequently, 50 µL of the concentrated extracts (ethanolic, aqueous, and infusion) was introduced solely into the first well of each row. Serial twofold dilutions (1/2) were then performed by successively transferring 50 µL from one well to the next, mixing thoroughly at each step. To ensure a constant final volume across all wells, 50 µL was discarded from the last well.

Following the dilution process, each well was inoculated with 50 µL of a standardized bacterial suspension adjusted to a 0.5 McFarland turbidity standard, resulting in a total working volume of 100 µL. To validate the assay, a negative control (broth + bacterial strain, without extract) and a positive control (broth + bacterial strain + antibiotic) were included.

The microplates were then incubated at 37 °C for 18 to 24 hours. Following this initial incubation, 10 µL of bromothymol blue was added to each well, followed by a second incubation for 2 hours at 37 °C. The MIC was defined as the lowest concentration of the extract that completely inhibited visible bacterial growth, indicated by the absence of turbidity in the medium.

To determine the MBC, 10 µL aliquots from the wells showing no visible growth during the MIC assay were subcultured onto Mueller-Hinton agar (MHA) plates using the spread-plate technique. After incubation at 37 °C for 24 hours, the MBC was defined as the lowest extract concentration that permitted no viable bacterial colony growth on the agar surface **(Boussayed,M *et al*.2025).**

Chapter IV :

Results and Discussion.

IV.1 Extraction Yield:

The extraction yields for the six extracts were calculated and are presented in **Table 5.**

Table 5 :characteristics and extraction yields of the studied green tea samples.

Samples	Extracts	Plant Material Mass (g)	Color / Appearance	Extract Weight (g)	Extraction Yield (%)
El-Berrad	**Infusion.**	5g	Dark green / Resinous	2.185g	43.7%
	Aqueous extract	5g	Dark green / Resinous	2.66g	53.30%
	Ethanolic extract.	5g	Dark green / Resinous	2.07g	47.64%
El-Rakim	**Infusion.**	5g	Dark green / Resinous	1.85g	34.14%
	Aqueous extract	5g	Dark green / Resinous	2.29g	48.38%
	Ethanolic extract.	5g	Dark green / Resinous	2.38g	41.50%

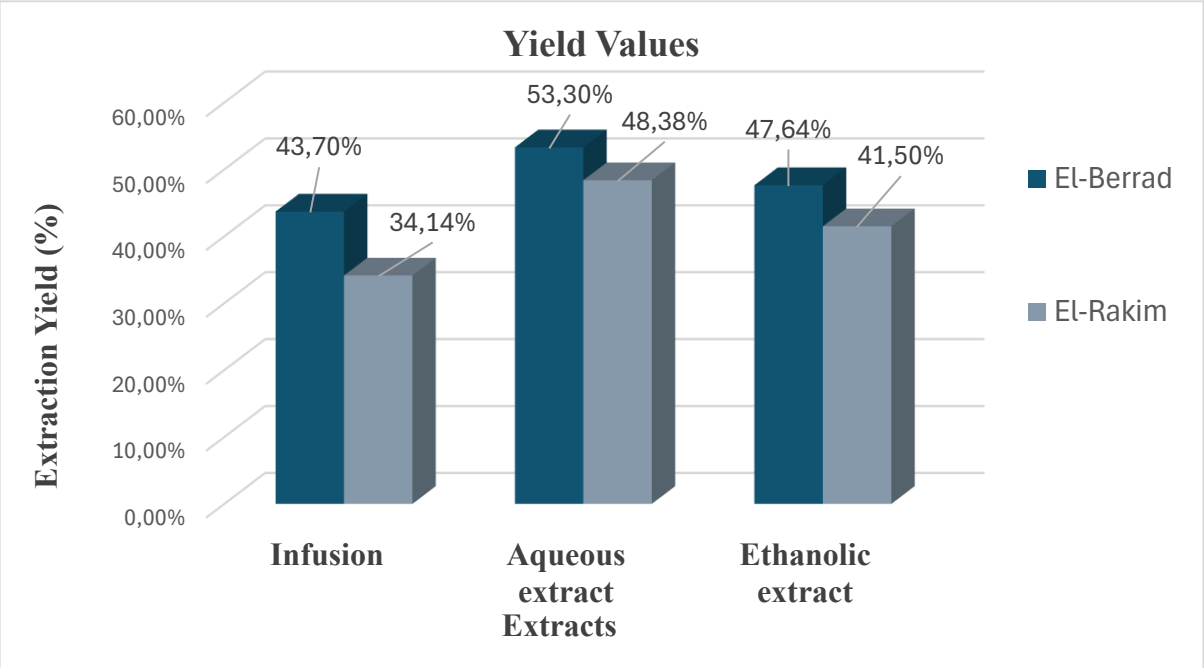


Figure 31: Comparative histogram of extraction yields (%) for the two green tea brands (El-Berrad and El-Rakim) using different extraction methods.

According to the results illustrated in Figure 31, the extraction yield values for both brands are relatively comparable. For the 'El-Berrad' brand, the infusion yield was 43.70%, while the ethanolic extract reached 47.64%. Notably, the aqueous extract exhibited the highest yield among all samples at 53.30%. In contrast, the 'El-Rakim' brand recorded its lowest yield for the infusion at 34.14%, whereas the aqueous and ethanolic extracts yielded 48.38% and 41.50%, respectively. Overall, it is observed that El-Berrad consistently provided higher yield values across all extraction methods compared to El-Rakim.

Based on the results, the aqueous extract provided the highest yield for both brands; however, El-Berrad demonstrated a slightly higher yield compared to El-Rakim.

Our findings are in perfect agreement with the study of **(Bushra Sultana *et al.*2009)**, who reported that variations in extract yields are primarily influenced by the vigor of the extraction procedure as well as the nature of the plant samples. Furthermore, the efficiency of the extracting solvent plays a crucial role in dissolving and recovering the endogenous bioactive compounds responsible for the plant's biological activities.

Regarding the extraction technique, Bushra Sultana et al. demonstrated that the yield is highly dependent on the method used, showing that extraction under reflux gives the best results. This strongly supports our experimental data, where both aqueous and ethanolic extracts exhibited the highest yields using this specific technique. They explained that hot solvent systems under reflux are significantly more efficient for recovery, regardless of the plant material or solvent involved.

This principle perfectly justifies our results, where the aqueous extract outyielded the ethanolic one. Since water possesses a higher polarity than ethanol, its extraction capacity for polar components was further enhanced under hot reflux conditions, leading to a higher yield. This phenomenon is also supported by **(Shon et al.2004)** , who confirmed that hot water and methanol systems are highly efficient for extracting antioxidant compounds.

IV.2 Phytochemical Screening of Green Tea Extracts :

Phytochemical screening is a qualitative analysis used to detect the chemical constituents present in *Camellia sinensis* plant. This method is based on visual observations, such as

color changes, precipitate formation, and turbidity. The screening results for the extracts of both tea brands (El-Berrad and El-Rakim) are summarized in the table below.

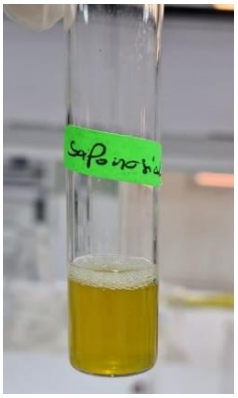
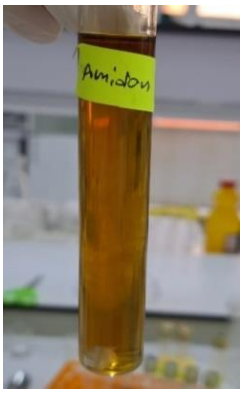
Table 6: Results of the phytochemical screening of the studied green tea extracts (El-Berrad and El-Rakim).

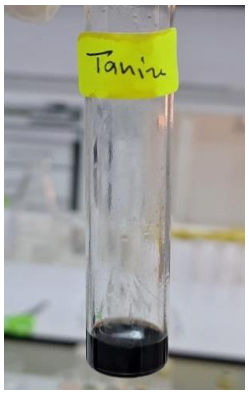
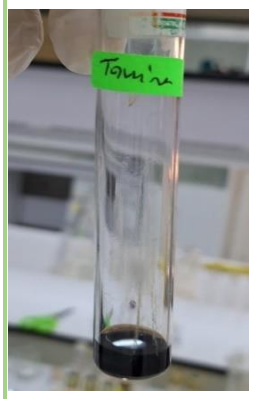
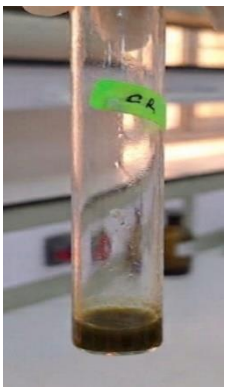
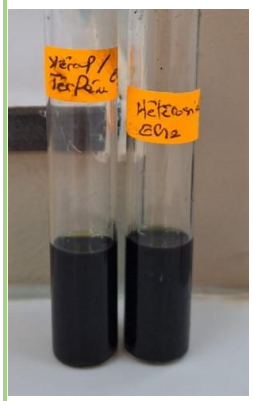
Phytochemical assay	**El-Berrad.**	**El-Rakim.**
Saponins.	+	+
Anthocyanins.	–	–
Starch.	–	–
Flavonoids.	+ +	+ +
Tannins.	+ + +	+ + +
Reducing compounds.	–	–
Sterols and terpenes.	+ + + Sterol.	+ + + Sterol.
Coumarins.	+	+
Alkaloids Mayer.	+ +	+ +
Alkaloids Wanger.	+ + +	+ + +

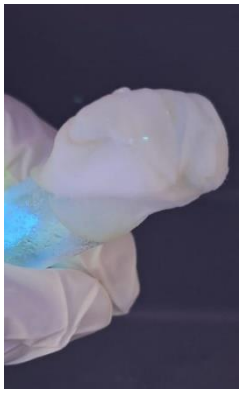
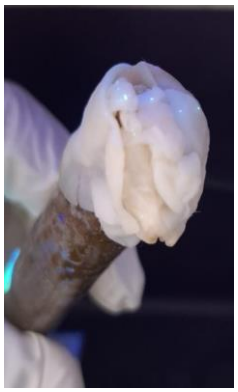
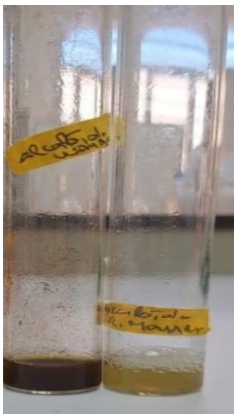
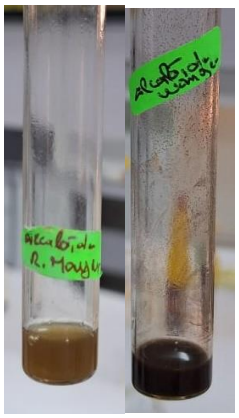
(+) : Presence;(++) : Strong presence (+++) : Very strong presence

(-): Absence.

Table 7: Summarized results of the phytochemical tests and visual observations for the two studied green tea extracts.

Phytochemical assay	**El-Berrad**	**El-Rakim**	**Observations**
Saponins.			For both brands, a very slight foam (less than 1 cm) appeared after shaking and remained stable after 15 minutes, indicating a positive result for saponins.
Anthocyanins.			Absence of a reddish-pink color, and the solution did not turn bluish-violet after waiting, indicating the absence of anthocyanins.
Starch.			Absence of a bluish-violet color, indicating the absence of strach.

Flavonoids.			Appearance of a faint reddish-pink color; however, it was not distinct due to the insufficient mass of magnesium turnings.
Tannins.			The appearance of a bluish-black color indicates the presence of gallic tannins.
Reducing compounds.			The solution remained greenish-brown with no formation of a brick-red precipitate, indicating a negative result for reducing compounds.
Sterols and terpenes.			The appearance of a green color in the first test indicates the presence of sterols. The appearance of a bluish-green color in the second test confirms the presence of steroidic heterosides.

Coumarins.			The appearance of very small spots under ultraviolet (UV) light indicates a trace amount of coumarins.
Alkaloids.			A reddish-brown precipitate was observed in Wagner's test, while Mayer's test showed moderate, non-intense turbidity.

As shown in Table 6, the phytochemical study of both green tea brands indicates that they are rich in bioactive compounds, such as tannins, flavonoids, alkaloids, and sterols. In contrast, coumarins and saponins are present in low quantities. Furthermore, anthocyanins, starch and reducing compounds were found to be absent in both brands, demonstrating that the results are identical for both samples.

The results obtained in this study are in agreement with those reported by **Amrane *et al*.2023**. As highlighted in his research, and supported by the findings of **Lateef** and **Lee *et al***, the presence of alkaloids, flavonoids, and tannins in green tea extracts enhances its biological effectiveness, particularly as an antioxidant among other therapeutic functions.

The findings of our study are further supported by the work of **AD Nasrine *et al*.2019**, which confirmed the presence of the same phytochemical families identified in our analysis.

This strong correlation across multiple studies reinforces the reliability of our results regarding the chemical composition of the studied green tea brands.

Comparing the results of the two brands, they are perfectly identical with no observed differences. This indicates that both samples contain the same secondary metabolites and exhibit the same biological activity. Consequently, it can be concluded that both samples possess the same level of quality.

IV.3 In vitro biological activities of Camellia sinensis extracts :

IV.3.1 Antioxidant activity:

IV.3.1.1 DPPH free radical scavenging assay:

The antioxidant activity of the various extracts from the two tea brands was evaluated by measuring their inhibitory capacity in a DPPH methanolic solution at a wavelength of 517 nm.

Ascorbic acid was used as a standard to verify the effectiveness of the experiment and for comparison.

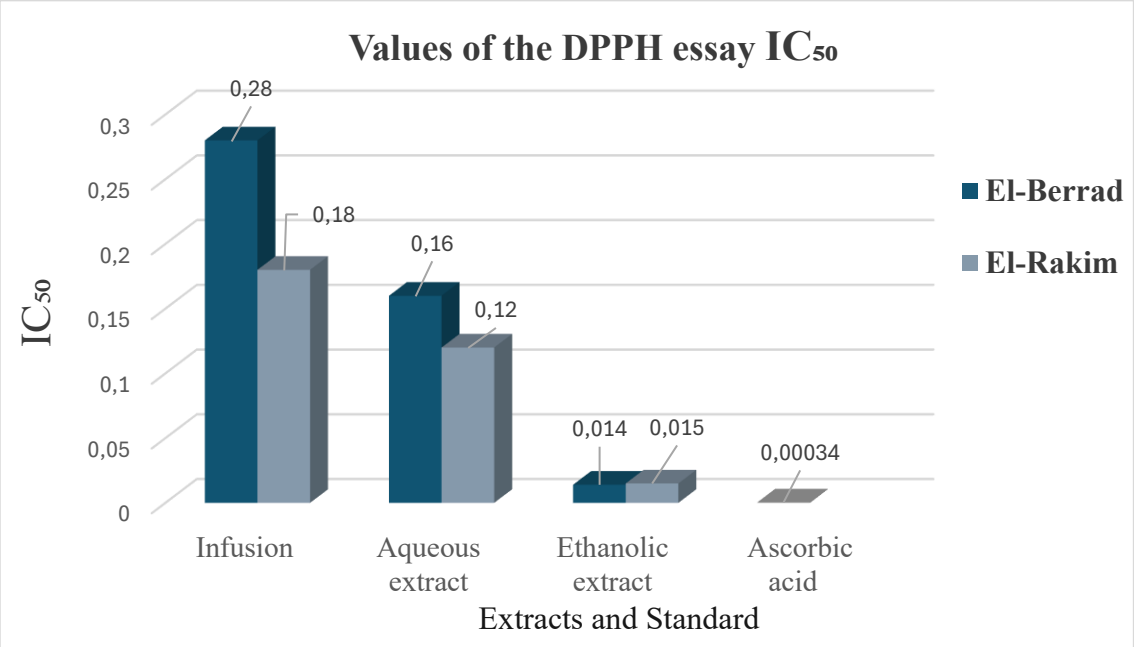


Figure 32: Histogram of DPPH antioxidant activity results for the tea extracts of both brands.

Figure 33 Illustrative photo of the DPPH assay results for the tea extracts of both brands.

The figure 31 displays the IC_{50} values for the three green tea extracts from both brands (El-Berrad and El-Rakim), along with Ascorbic acid as a standard.

The IC_{50} is defined as the concentration required to inhibit 50% of free radicals; thus, a lower value indicates higher antioxidant activity.

Among all tested samples, the ethanolic extract of El-Berrad exhibited the highest antioxidant activity with a value of IC_{50} 0.014 mg/mL, which is the lowest value among the extracts. In contrast.

The infusion of El-Berrad showed the lowest antioxidant activity with an IC_{50} value of 0.28 mg/mL, representing the highest recorded value in the study; conversely, the infusion of El-Rakim demonstrated a superior performance with a significantly lower IC_{50} value (0.18mg/ml) , indicating a better effect than that of the first sample's infusion.

Regarding the aqueous extracts, El-Rakim demonstrated very good activity with a value of IC_{50} 0.12 mg/mL, which was superior to the value of IC_{50} 0.16 mg/mL recorded for El-Berrad.

Overall, the ethanolic extracts provided the most potent antioxidant effects with very close values of IC_{50} 0.014 mg/mL and 0.015 mg/mL.

In an overall comparison between the two samples, the (El-Rakim) extracts demonstrated superior antioxidant performance over the (El Berrad) extracts in most methods, with the sole exception of the ethanolic extract. In the latter, 'El Berrad' showed slightly higher activity, although the difference was negligible.

The order of the tested extracts is as follows :

Ethanolic extract (El Berrad) > Ethanolic extract(El - Rakim) > Aqueous extract (El - Rakim) > Aqueous extract(El Berrad) > Infusion (El - Rakim) > Infusion (El Berrad).

The tested standard, Ascorbic acid, exhibited a significantly lower IC_{50} value compared to all the extracts, with a value of 0.00034 mg/mL. This result serves as a strong evidence of the reliability and effectiveness of the assay, confirming the accuracy of the experimental procedure used to evaluate the antioxidant activity.

The results obtained for the ethanolic extract in this study are in high agreement with the findings of **(Benfedda Darine *et al.*2025)** , who reported an IC_{50} value of 14.62 ± 0.51 µg/mL for the methanolic extract of green tea. Furthermore, our results are comparable to those of **(Nazliniwaty *et al.*2020)** , who achieved an IC_{50} value of 11.83 ± 0.005 µg/mL. This correlation demonstrates the exceptional bioactive efficiency of this plant, particularly the ethanolic extract, which successfully recovered phenols and catechins with high precision. According to **(Ahmed *et al.*2026)**, the superior performance of ethanol and methanol is attributed to their nature as polar solvents, offering high solubility and excellent extraction capacity.

The lower antioxidant activity observed in the aqueous extracts compared to the ethanolic ones can be attributed to the nature of the solvent. As reported by **(Ahmed *et al*. 2025)** , water has limited efficiency in extracting hydrophobic compounds. Despite

this, the results for the aqueous extracts remained significant, confirming the rich phenolic content of the studied samples.

The disparity between Decoction (boiled) and Infusion (steeped) values can be explained by the varying extraction conditions, specifically temperature and duration. These findings align with the comparative study by **(L.E Ramírez-A. *et al*.2017)** , which demonstrated that tea extraction at different temperatures leads to variations in yield and chemical composition. Their study found that infusions prepared at low temperatures (20-40°C) exhibited weaker activity than those prepared at higher temperatures (60-100°C). In our study, the superior performance of the 'Decoction' method is due to the prolonged boiling time (one hour) at 100°C, which facilitated a more exhaustive recovery of polyphenols and catechins, confirming that the extraction process is strictly time and temperature-dependent.

The disparity in the recorded values between the two samples can also be explained by referring to the study of **(Mitra Mehrabani *et al*.2022)**. This study highlighted that variations in antioxidant activity are primarily attributed to the influence of climate, cultivation methods, and preparation processes, as well as the geographic location. All these factors significantly impact the quality and concentration of polyphenols in tea leaves. Consequently, the differences observed in our results may reflect disparities in sample quality arising from their geographic origin or the processing conditions prior to commercialization.

IV.3.1.2 Ferric Reducing Antioxidant Power (FRAP) Activity :

The evaluation of the reducing capacity of the investigated extracts is based on their ability to reduce the yellowish ferric ions (Fe^{3+}) and convert them into blue-green ferrous ions (Fe^{2+}) This color intensity is monitored via spectrophotometric absorbance measurements at a wavelength of 700 nm.To quantitatively estimate this activity, the effective concentration values required to achieve an absorbance of 0.5 ($A_{0.5}$) were

calculated and directly compared with the reference standard used, namely ascorbic acid. The experimental data presented below summarize the final outcomes of these assays:

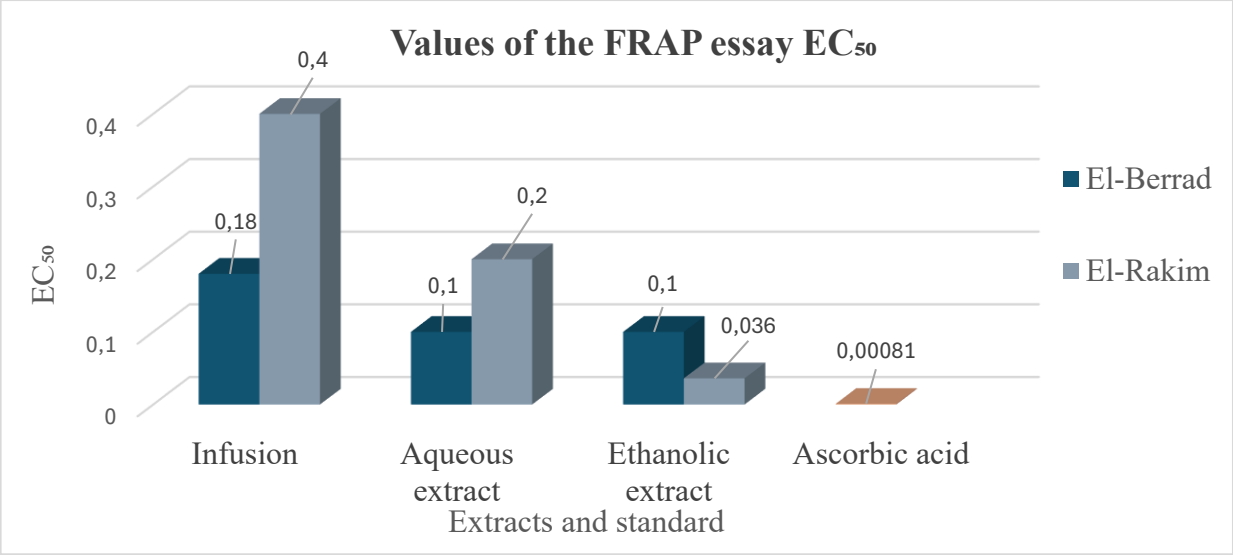


Figure 36: Histogram of FRAP antioxidant activity results for the tea extracts of both brands.

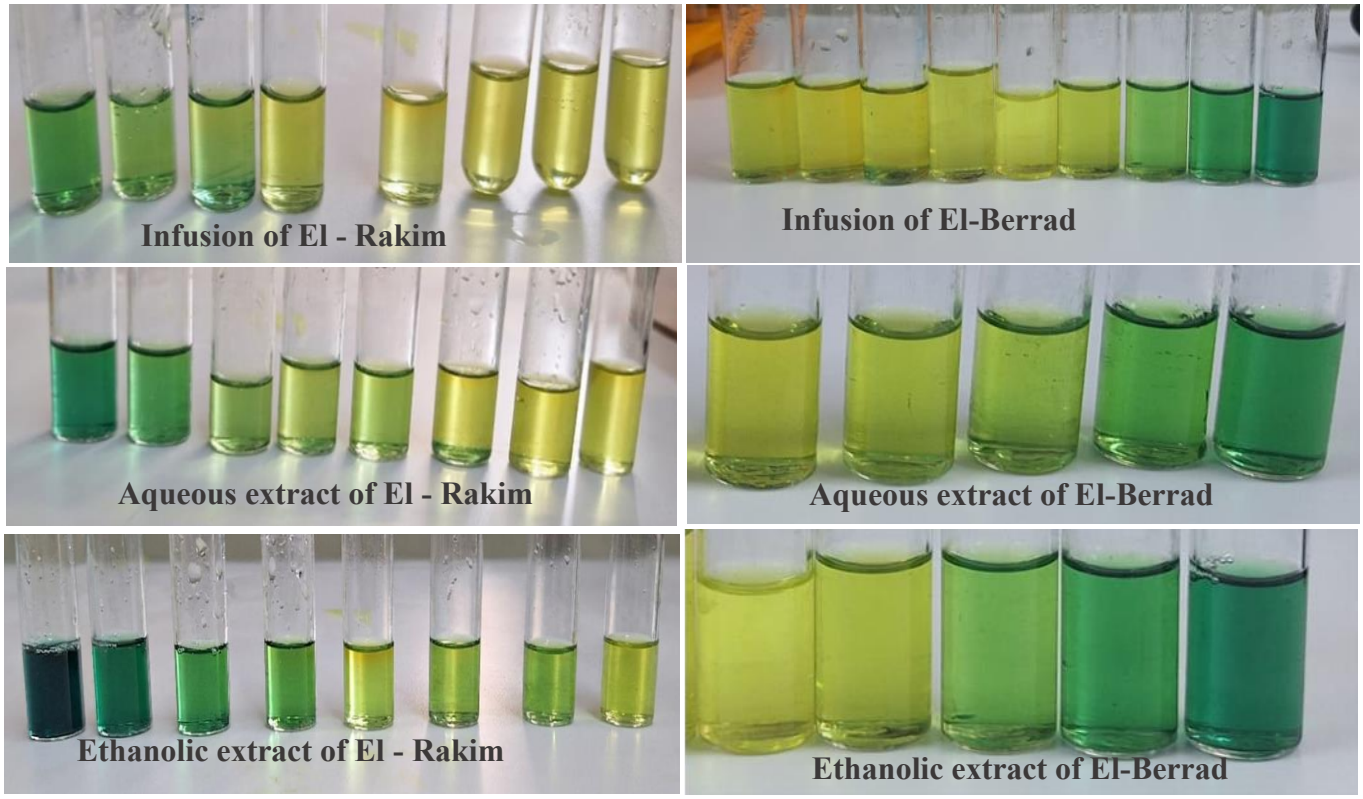


Figure 35: Illustrative photo of the FRAP assay results for the tea extracts of both brands .

Based on the obtained results and the EC_{50} histogram, the ethanolic extract of the 'El -Rrakim' sample demonstrated an exceptional and prominent reducing capacity, exhibiting the lowest EC_{50} value (0.036 mg/mL). This indicates that the ethanolic fraction of 'El-Rakim' possesses the highest efficiency and a superior capacity to reduce ferric iron (Fe^{3+}) compared to all other investigated extracts. Conversely, the aqueous infusion of the same brand 'El-Rakim' recorded the highest EC_{50} value (0.40 mg/mL), indicating a lower reducing power relative to the remaining fractions, followed by its decoction (boiling water extract) which showed a moderate and satisfactory efficiency with a value of (0.20 mg/mL) .Regarding the 'El Berrad' sample, the aqueous infusion exhibited a good reducing capacity with an EC_{50} of (0.18 mg/mL).

Interestingly, both the aqueous decoction and the ethanolic extract of 'El Berrad' yielded identical, highly effective results with a value of 0.10 mg/mL . In a comprehensive comparison among the extraction solvents, the ethanolic extraction proved to be the most efficient technique overall, as clearly evidenced by the 'El -Rrakim' sample. This is followed by the decoction method for both brands, which demonstrated enhanced performance despite the noticeable variations between the two samples. Finally, the aqueous infusion exhibited the lowest comparative efficiency. Consequently, it can be concluded that while the 'El Berrad' brand generally surpassed 'El -Rrakim' ' across the aqueous extracts in this assay, the "El -Rrakim' ethanolic extract was remarkably superior, standing out as the most potent antioxidant fraction overall.

In a comparative assessment with previous literature using the same methodology (EC_{50} calculated at an absorbance of 0.5), the ethanolic extract of the 'Arrakim' sample demonstrated outstanding superiority and a potent reducing capacity. Our sample exhibited a significantly lower and preferred value of 36 µg /mL (equivalent to 0.036 mg/mL) compared to the findings reported by **(Benfedda.d *et al*.2025)** (56.56 ±0.63 µg /mL) and **(Amir *et al*. 2020)** (73.80 ± 2.1 µg /mL). This remarkable numerical lower value directly reflects the exceptional quality of the investigated green tea brand and its supreme efficacy in electron donation and ferric ion reduction at highly minimal concentrations relative to earlier studies.

IV.3.2 Anti-Inflammatory Activity :

IV.3.2.1 *In vitro* Anti-inflammatory Activity by HRBC Membrane Stabilization Method:

The *in vitro* anti-inflammatory activity of green tea extracts was evaluated using the Human Red Blood Cell (HRBC) membrane stabilization assay according to a validated protocol, with Ibuprofen serving as the reference standard (positive control). Furthermore, a single screening concentration of **5 mg/mL** was tested to initially verify and detect the presence of anti-inflammatory activity within these extracts.

This assay is strictly based on measuring the efficiency of the extracts in protecting red blood cells from lysis induced by hypotonic solution stress. The density of hemoglobin released due to cell lysis was quantified spectrophotometrically at a wavelength of 560 nm, and the percentage of membrane protection was subsequently calculated as a direct indicator of the anti-inflammatory potency. The results are illustrated below:

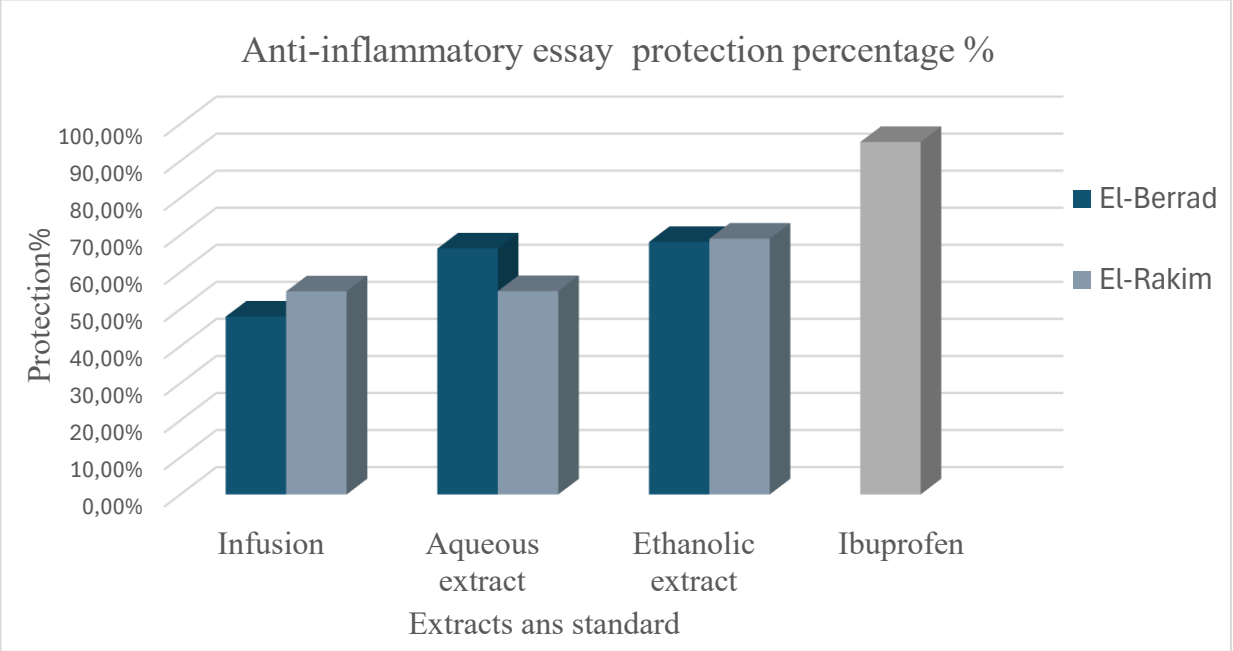


Figure 37: Histogram of anti-inflammatory activity results for the tea extracts and the positive control.

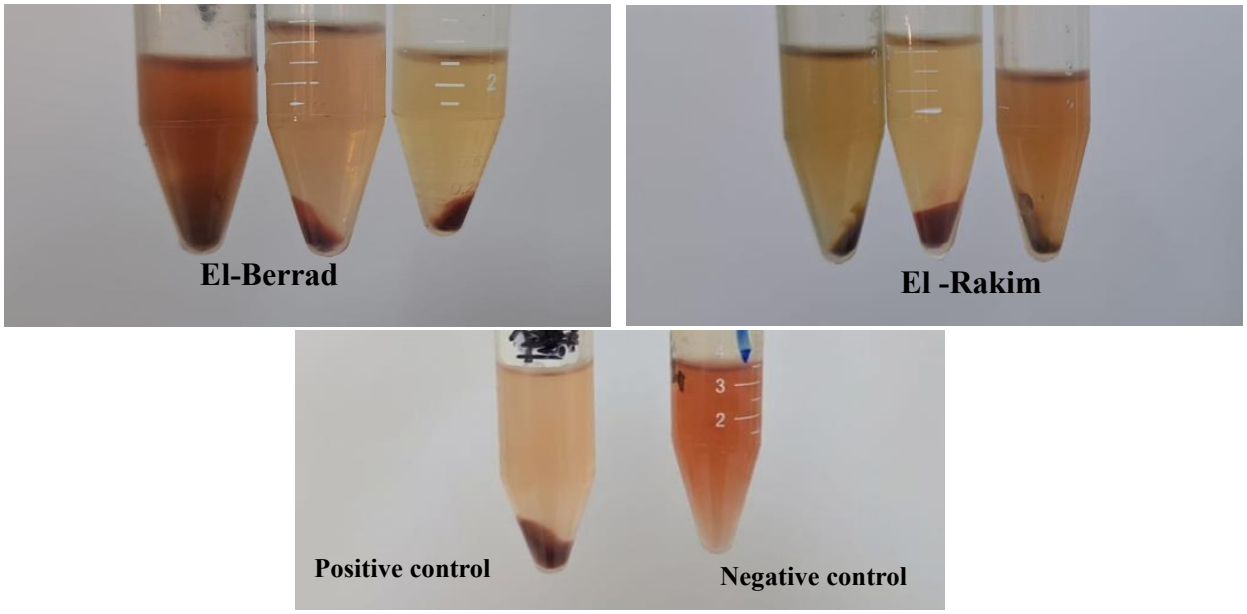


Figure 38: Anti-inflammatory activity results of the tea extracts, positive control, and negative control.

Histogram 36 illustrate the erythrocyte membrane protection percentages exerted by the various tea extracts for both samples.

According to the obtained data, the highest protection rate was recorded by the ethanolic extract of the "El-Rakim" sample at 69.03%, indicating a very potent *in vitro* anti-inflammatory activity. Conversely, the lowest value was observed in the infusion of the "El-Berrad" sample at 48.67%. Interestingly, the decoction of the "El-Berrad" sample showed a remarkable protective efficiency of 66.37%. Regarding the infusion and decoction of the "El-Rakim" sample, they exhibited almost identical values, reaching 54.81% and 54.87%, respectively. Finally, the ethanolic extract of "El-Berrad" scored 68.14%, which is highly comparable to the second brand, further confirming the robust potential of these extracts.

As a general comparison, all investigated extracts demonstrated a high stabilizing efficiency despite numerical variations. This variance is strictly attributed to the difference in extraction solvents and processing conditions. In this assay, no single sample can be considered superior to the other; the results are closely contiguous, with each extract showing a distinct advantage based on its preparation method.

Furthermore, the high value recorded by Ibuprofen (95.13%), a well-known standard non-steroidal anti-inflammatory drug, confirms the validity and success of this experimental protocol, serving as a reliable benchmark to validate the efficacy of our extracts.

In this context, our experimental findings demonstrate a remarkable alignment with the study conducted by **(Akanksha Singh *et al*. 2025),** where the inhibition rate reached approximately 60%. The authors highlighted that tea compounds possess a distinctive dual efficiency as simultaneous antioxidant and anti-inflammatory agents, which strongly corroborates the high antioxidant results observed in our work. Furthermore, as indicated by **(Franklin Pacheco-Coello *et al*.2021)**, the antioxidant capacity is intimately linked to the anti-inflammatory response, a relationship clearly manifested in our study where the erythrocyte membrane protection rate reached 69.03%.

From a biological standpoint, oxidative stress and inflammation are closely contiguous and interconnected processes within the body; thus, shielding cells from oxidative damage via tea secondary metabolites serves as a direct and essential pathway to suppress inflammatory cascades. This is supported by previous literature emphasizing that the antioxidant capacity of green tea phenolic compound s plays a pivotal role in their anti-inflammatory potency.

Specifically, epigallocatechin gallate (EGCG), the principal component of green tea, is well-known for its capacity to downregulate the gene expression of pro-inflammatory enzymes, such as cyclooxygenase-2 (COX-2) and nitric oxide synthase (NOS), by blocking the activation of the nuclear factor NF-κB **(Franklin Pacheco-Coello *et al*.2021).**

Consequently, these referenced studies collectively summarize, support, and scientifically elucidate the integrity and correlation of our obtained data, validating the promising biomedical value of our extracts in managing oxidative stress and chronic inflammatory disorders.

IV.3.3 Antimicrobial activity :

In order to evaluate the antimicrobial activity of the extracts from both green tea samples, screening was conducted against six pathogenic strains(*Escherichia coli, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis*), including four bacterial and two fungal strains(*Penicillium zacinthae, Aspergillus niger*), This section aims to determine the inhibitory potential of our various extracts and to compare their efficiency against these target micro-organisms.

IV.3.3.1 Antibacterial activity:

The in vitro antibacterial efficiency of three distinct extracts (ethanolic, aqueous, and infusion) prepared from two commercial Algerian brands of green tea (*Camellia sinensis*) was evaluated using the agar well diffusion method on Mueller-Hinton medium. The antibacterial potency was determined by measuring the diameter of the inhibition zones (in millimeters) around the extract-loaded wells following an incubation period of 18 to 24 hours at 37°C. The inhibitory activity was screened against four pathogenic bacterial strains: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Bacillus cereus*, the results and discussions of which are detailed below:

Table 8: Inhibition zones of the infusion extract for both tea brands against the bacterial strains

		Sample 1: El-Berrad				Sample 2: El-Rakim			
Extract	**Concentrations**	**E. coli**	**S. aureus**	**B. subtilis**	**B. cereus**	**E. coli**	**S. aureus**	**B. subtilis**	**B. cereus**
Infusion	35 mg/ml	6 mm	9 mm	7 mm	6 mm	6 mm	9 mm	8 mm	7mm
	25 mg/ml	6 mm	8 mm	7 mm	6 mm	6 mm	7mm	8 mm	6 mm
	15 mg/ml	6 mm	6 mm	7mm	6 mm	6 mm	6 mm	7 mm	6 mm
	5 mg/ml	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm

Table 9: Inhibition zones of the Aqueous extract for both tea brands against the bacterial strains

		Sample 1: El-Berrad				Sample 2: El-Rakim			
Extract	**Concentrations**	**E. coli**	**S. aureus**	**B. subtilis**	**B. cereus**	**E. coli**	**S. aureus**	**B. subtilis**	**B. cereus**
Aqueous extract.	35 mg/ml	6 mm	11 mm	11 mm	10mm	6 mm	10 mm	10 mm	8 mm
	25 mg/ml	6 mm	10 mm	10mm	8mm	6 mm	9 mm	8 mm	6 mm
	15 mg/ml	6 mm	8 mm	9 mm	6 mm	6 mm	6 mm	7 mm	6 mm
	5 mg/ml	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm

Table 10: Inhibition zones of the Ethanolic extract for both tea brands against the bacterial strains.

		Sample 1: El-Berrad				**Sample 2: El-Rakim**			
Extract	**Concentrations**	***E. coli***	***S. aureus***	***B. subtilis***	***B. cereus***	***E. coli***	***S. aureus***	***B. subtilis***	***B. cereus***
Ethanolic extract.	35 mg/ml	6 mm	16 mm	15mm	13mm	6 mm	15mm	14 mm	11 mm
	25 mg/ml	6 mm	11 mm	13 mm	12mm	6 mm	13mm	11 mm	11 mm
	15 mg/ml	6 mm	7mm	9 mm	10mm	6 mm	7 mm	10 mm	10 mm
	5 mg/ml	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm	6 mm

Table 11: Antibiotic results against bacterial strains.

Antibiotics / Bacterial strains	**Gentamicine 200 µg**	**Erythromycine 60 µg**	**Amoxicilline 30 µg**	**Vancomycine 05 µg**	**Rifampicine 30 µg**	**Pénicilline 10 µg**
E. coli	23 mm	09 mm	25 mm	00	09 mm	05 mm
S. aureus	27 mm	37 mm	42 mm	13 mm	35 mm	43 mm
B. cereus	25 mm	19 mm	07 mm	11 mm	13 mm	00
B. subtilis	25 mm	19 mm	07 mm	09 mm	15 mm	00

Table 12: Photographic arrangement of El-Berrad tea extracts at concentrations of 25 mg/mL and 35 mg/mL against the bacterial strains.

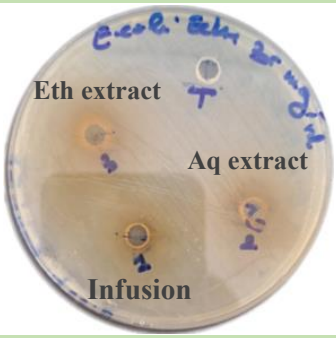
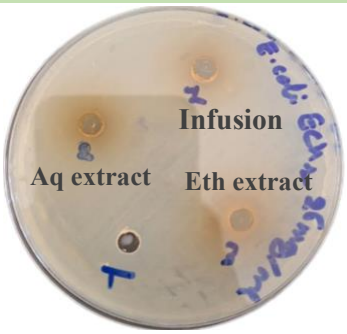
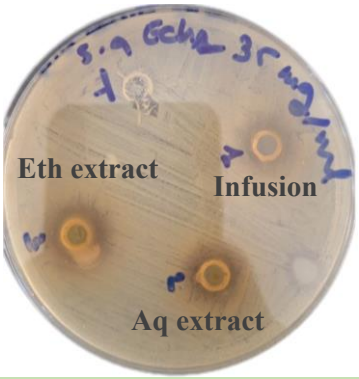
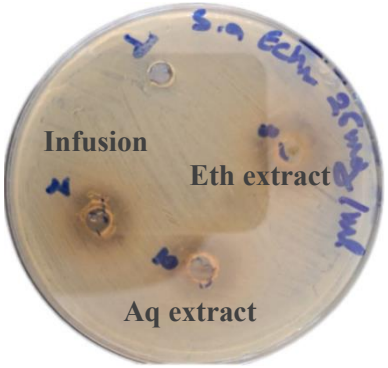
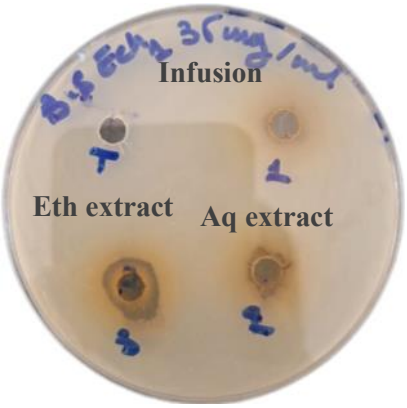
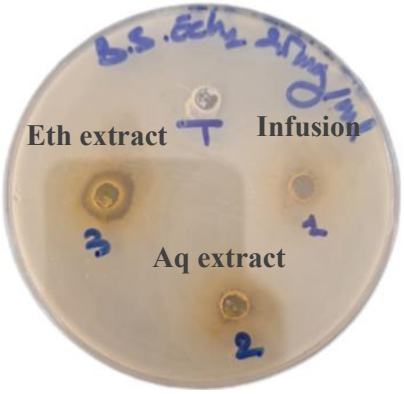
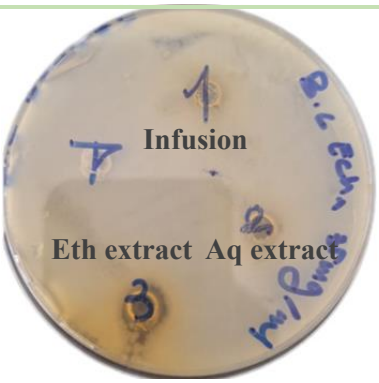
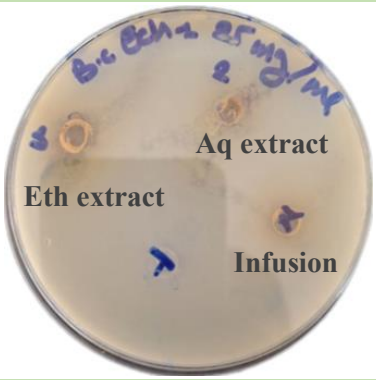
Conc / Bacterial strains	35mg/ml.	25mg/ml.
E. coli		
S. aureus		
B. subtilis		
B. cereus		

Table 13: Photographic arrangement of El-Raqim tea extracts at concentrations of 25 mg/mL and 35 mg/mL against the bacterial strains.

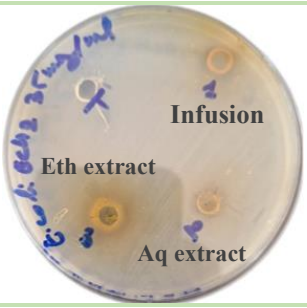
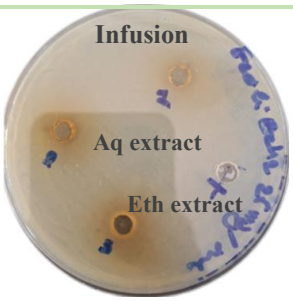
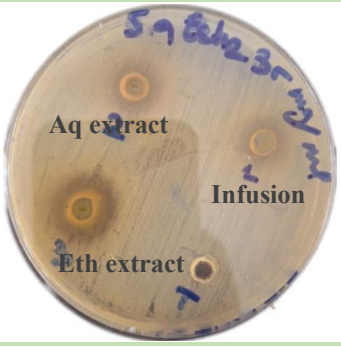
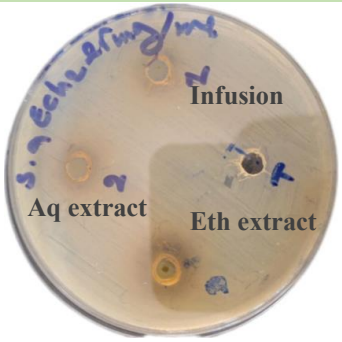
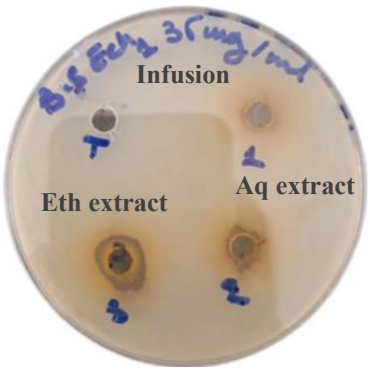
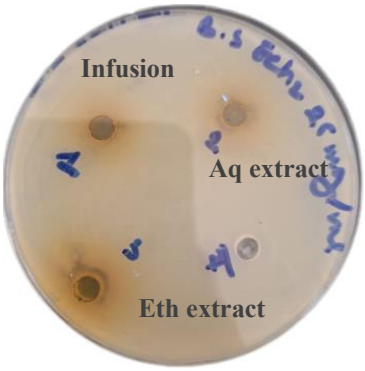
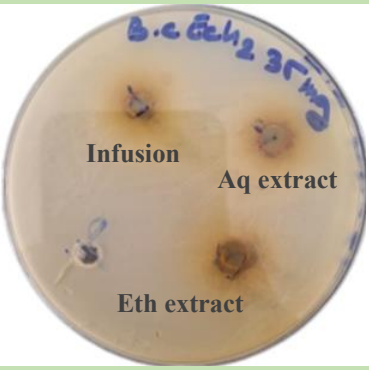
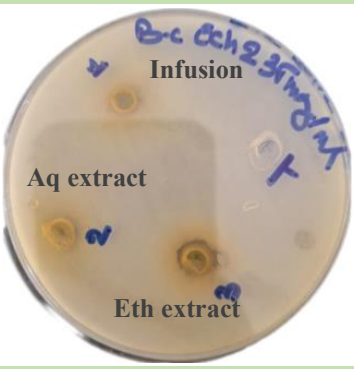
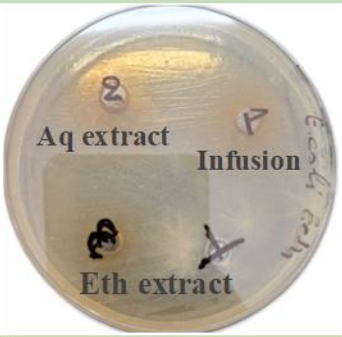
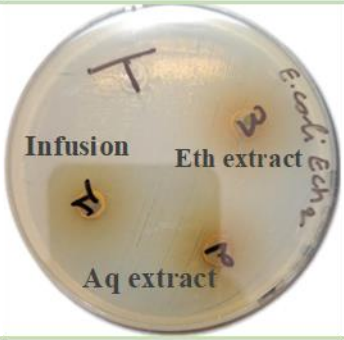
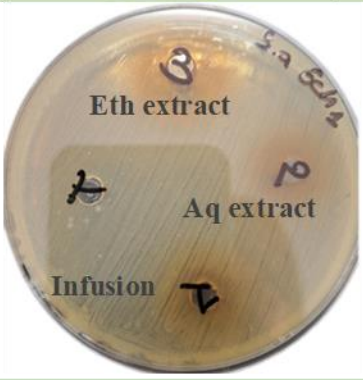
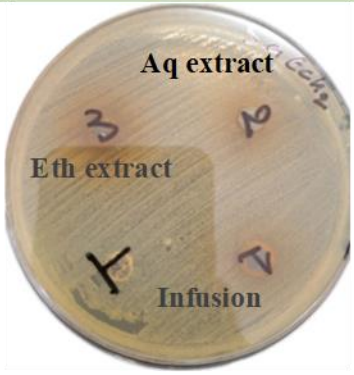
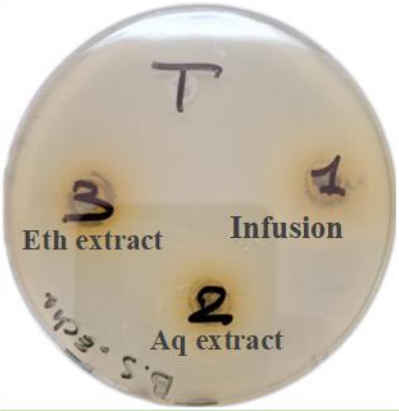
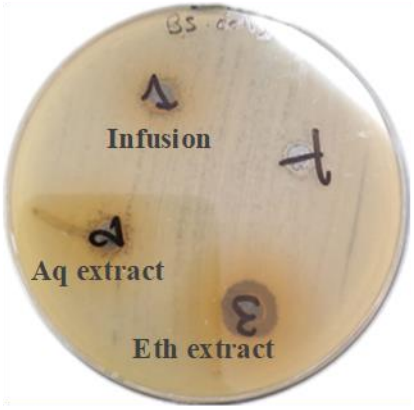
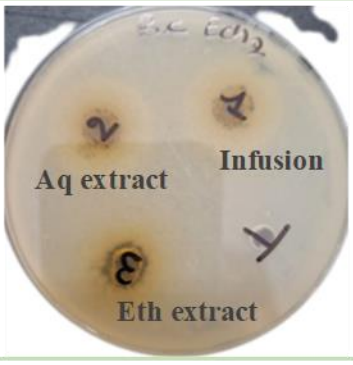
Conc / Bacterial strains	35mg/ml.	25mg/ml.
E. coli		
S. aureus		
B. subtilis		
B. cereus		

Table 14: Photographic arrangement of both tea brands at a concentration of 15 mg/mL against the bacterial strains

	15mg/ml	
Bacterial strains	Sample 1: El-Berrad	Sample 2: El-Rakim
E. coli		
S. aureus		
B. subtilis		
B. cereus		

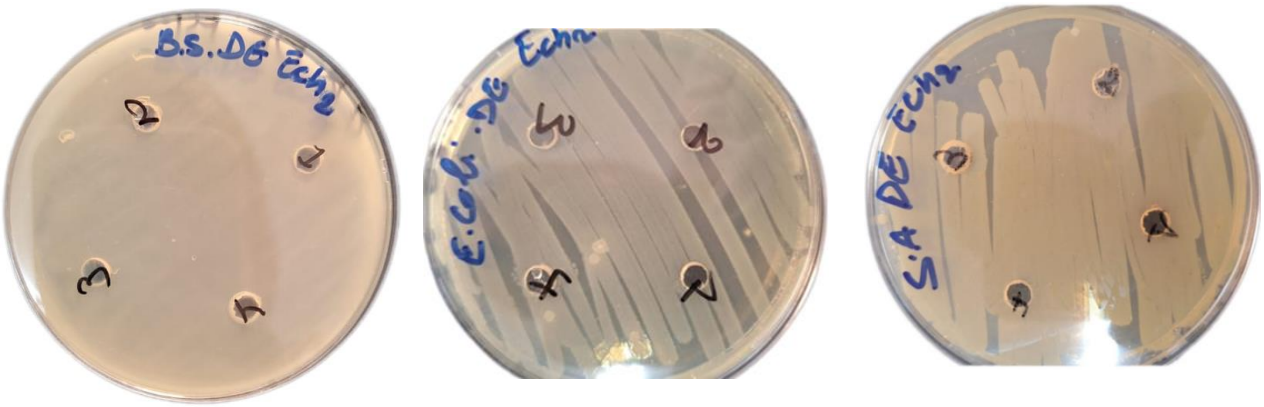


Figure 40: Illustrative photos showing the absence of inhibition zones for the tea extracts at a concentration of 5 mg/mL against the bacterial strains.

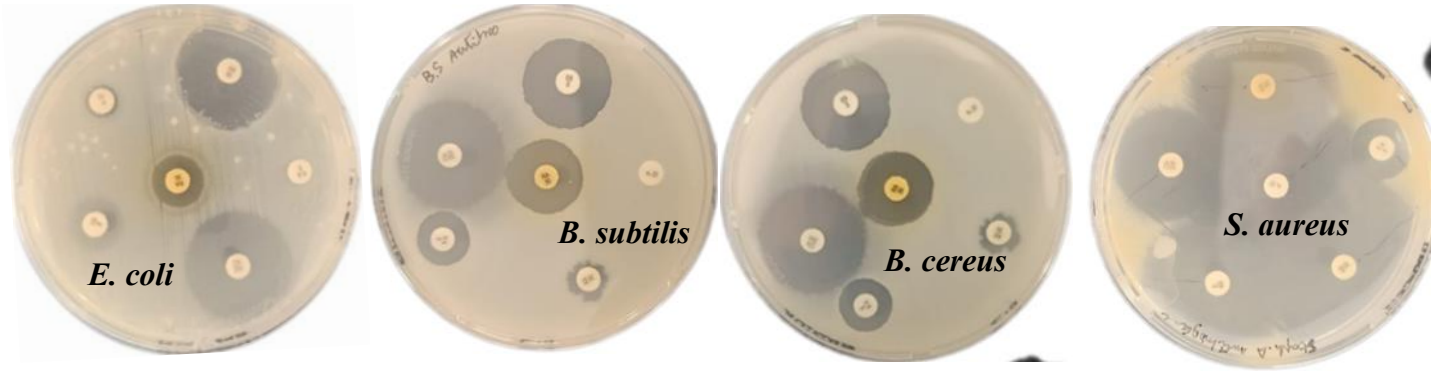


Figure 39: Antibiotic results against bacterial strains.

The results illustrated in Table8, which are photographically documented within the comprehensive image plate presented in **Tables 12,13 and 14** represent the inhibition zone diameters of the infusion extracts from both green tea samples (El-Berrad and El-Raqim) against the four bacterial strains.

It was observed that *Escherichia coli* exhibited no zone of inhibition for either sample, indicating that this strain is highly resistant and the extract's efficacy against it is negligible

Regarding *Staphylococcus aureus*, inhibition zones appeared only at the strongest concentrations (35 and 25 mg/mL). The recorded diameters were very close and weak for both samples, reaching 9 mm and 8 mm for the El-Berrad sample, and 9 mm and 7 mm for the El-Raqim sample, respectively, which reflects a very low antibacterial activity.

For *Bacillus subtilis*, similar low inhibition diameters were observed at the three strong concentrations (35, 25, and 15 mg/mL), remaining at 7 mm for El-Berrad, and around 8 to 7 mm for El-Raqim. These values are considered very weak compared to the positive control.

As for the fourth strain, *Bacillus cereus*, it showed total resistance to the El-Berrad infusion across all concentrations. Conversely, the El-Raqim sample induced an extremely weak inhibition zone of 7 mm only at the highest (strongest) concentration of 35 mg/mL (given that the well diameter is 6 mm), while no effect was observed at lower concentrations (particularly at 5 mg/mL) **(Figure 38).**

In conclusion, the infusion extract does not possess strong antibacterial efficacy against the studied strains at the current tested concentrations.

As photographically documented within the comprehensive image plate in **Table 9,12,13 and 14** details the antibacterial efficacy of the decoction extracts from both green tea samples (El-Berrad and El-Raqim) against the four bacterial strains.

Overall, the decoction yielded relatively higher inhibition results compared to the infusion extract. Nevertheless, *Escherichia coli* remained completely resistant, showing a total absence of inhibition zones across all concentrations. Furthermore, the lowest concentration (5 mg/mL) failed to exhibit any antibacterial activity against any of the tested strains **(Figure 38).**

Regarding *Staphylococcus aureus*, a noticeably improved performance was observed compared to the infusion, with closely matching diameters between both commercial brands. For the El-Berrad sample, inhibition was restricted to the strong concentrations (35 and 25 mg/mL), reaching diameters of 11 mm and 10 mm, respectively. Similarly, the El-Raqim sample displayed zones of 10 mm and 9 mm at the same concentrations. These values are classified as moderate, serving as a promising indicator of the extract's potency, despite being somewhat lower than the positive control.

For *Bacillus subtilis*, the results were identical to those of S. aureus for the El-Berrad sample, where inhibition zones appeared at the strong concentrations (35, 25, and 15 mg/mL) with diameters of 11 mm, 10 mm, and 9 mm, respectively. For El-Raqim, the activity was relatively lower than El-Berrad at the same concentrations, yielding diameters of 10 mm, 8 mm, and 7 mm. Although these results represent an improvement over the infusion, they remain generally modest.

Finally, for the fourth strain, *Bacillus cereus*, a notable variance occurred where El-Berrad outperformed El-Raqim, inducing inhibition zones at 35 and 25 mg/mL with diameters of 10 mm and 8 mm, respectively. Conversely, the El-Raqim extract demonstrated activity restricted only to the highest concentration of 35 mg/mL, with a weak diameter of 8 mm.

The results compiled in Table 9, which are photographically documented within the comprehensive image plate presented in **Table 10,12,13 and 14**, indicate that the ethanolic extract for both green tea samples demonstrated the highest and most superior antibacterial efficiency compared to the two previous aqueous extracts (decoction and infusion).

Regarding *Staphylococcus aureus*, an excellent inhibitory activity was observed for both samples. The inhibition zones extended across the three strong concentrations (35, 25, and 15 mg/mL), with diameters reaching (16, 11, and 7 mm) for the El-Berrad sample, and (15, 13, and 7 mm) for the El-Raqim sample, respectively. These closely matching values provide clear evidence of the high potency of this extract against this pathogenic strain.

For *Bacillus subtilis*, the results were also highly satisfactory, matching the S. aureus profiles for the El-Raqim sample and closely aligning with them for El-Berrad. The inhibition zones appeared at the strong concentrations (35, 25, and 15 mg/mL), yielding diameters of (15, 13, and 9 mm) for El-Berrad, and (14, 11, and 10 mm) for El-Raqim, thereby confirming the distinct efficacy of the ethanolic extract against it. As for *Bacillus cereus*, a relative superiority of El-Berrad over El-Raqim was noted, recording diameters of (13, 12, and 10 mm) at the three strong concentrations, compared to (11, 11, and 10 mm) for El-Raqim. These somewhat uniform values are classified as moderate, establishing *B. cereus* as the least sensitive Gram-positive strain tested.

Conversely, *Escherichia coli* maintained its absolute resistance, as inhibition zones were completely absent across all tested concentrations and extracts. Furthermore, the lowest concentration (5 mg/mL) failed to exhibit any antibacterial activity against any of the strains without exception **(Figure 38).**

These laboratory findings allow us to conclude that the ethanolic extract successfully penetrated and disrupted the cell wall of Gram-positive bacteria with strong, varying efficiency depending on the strain. In contrast, green tea exerted no antimicrobial effect on the Gram-negative bacterium (*E. coli*) within the utilized limits. This fundamental variation in sensitivity and resistance is deeply attributed to the structural differences in the cell wall between Gram-positive and Gram-negative bacteria. The latter possesses a robust outer membrane that acts as a physical and chemical barrier, effectively blocking the penetration of bioactive compounds into the cell.

This conclusion aligns with the findings reported by **(Omrane et al. 2025)** in his study on the ethanolic and aqueous extracts of green tea. He demonstrated that the antibacterial

mechanism is strictly dose-dependent, as his extracts showed no inhibition against E. coli except at very strong concentrations reaching 200 mg/mL (yielding diameters of up to 30 mm). He also confirmed the superiority of the ethanolic extract over the aqueous one in suppressing S. aureus, with diameters ranging between 20 and 28 mm. This justifies the absence of effect in our experiments at the low concentration (5 mg/mL) and highlights our future need for stronger concentrations matching those reported in the literature.

On a biochemical level, **(Tariq A.L *et al*.2012)**, in consensus with the data cited by **(Omrane et al.2025)**, point out that the broad-spectrum inhibitory effect of green tea is primarily driven by its rich content of catechins and polyphenols, most notably epigallocatechin gallate (EGCG) a bioactive compound with proven therapeutic efficacy against complex and resistant bacterial infections. These active phytochemicals interact through multi-targeted defense mechanisms: they induce severe and direct damage to the bacterial cytoplasmic membranes, while simultaneously disrupting the enzymatic machinery involved in bacterial DNA replication. This dual action effectively halts cellular division and restricts colony growth. Additionally, these biomolecules play a dynamic role in preventing the development and formation of bacterial biofilms, which pathogenic strains typically utilize for protection.

In conclusion, the superior performance of the ethanolic extract over the two aqueous extracts (decoction and infusion) proves that the variance in efficacy is closely linked to extraction conditions and the nature of the solvent used. Ethanol, as an organic polar solvent, possesses an outstanding capacity to dissolve and extract phenolic compounds and hydrophobic catechins, facilitating their penetration through biological membranes. This efficiency was thermally enhanced in our study through the reflux extraction process for a full hour. Conversely, pure water (in the decoction extract) failed to dissolve highly hydrophobic compounds despite utilizing the same boiling temperature, while the aqueous infusion showed the weakest performance due to the lower temperature and reduced contact time.

IV.3.3.2 Antifungal Activity:

To highlight the antifungal potential of our prepared formulations, a comparative study was conducted to examine the effect of three distinct extracts (infusion, decoction, and ethanolic extract) obtained from two green tea brands (El-Berrad and El-Raqim) on the mycelial development of two pathogenic fungal strains, namely *Aspergillus niger* and *Penicillium zacinthae.*

The experimental protocol was executed on solid Sabouraud Dextrose Agar (SDA) medium using the agar well diffusion technique. This approach allowed us to evaluate the inhibitory capacity of the extracts by assessing the diameters of the inhibition zones (expressed in mm). The Petri dishes were subjected to a prolonged incubation period of 7 days under a controlled temperature of 28°C.

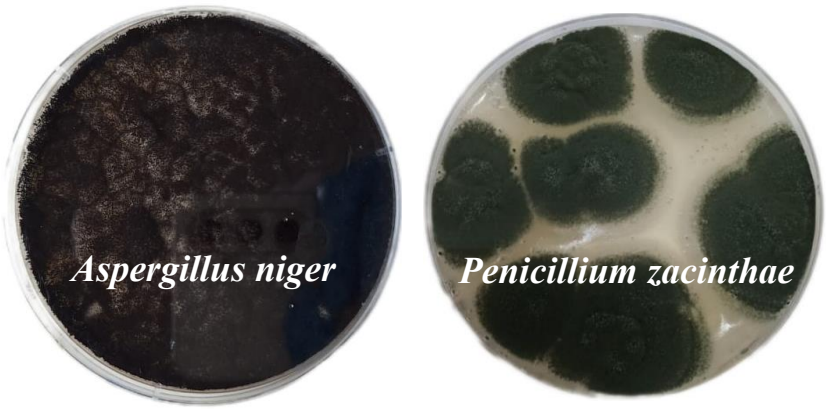


Figure 41: Antifungal activity results of the three tea extracts for both brands against *Aspergillus niger and Penicillium zacinthae*.

Based on the obtained laboratory results and as photographically documented within the comprehensive image plate presented in **Figure 40**, a completely negative outcome was recorded. Inhibition zones were entirely absent (0 mm) around all wells for all three extracts (ethanolic, decoction, and infusion) of both green tea brands (El-Berrad and El-Raqim) against the two tested fungal strains (*Aspergillus niger and Penicillium zacinthae*) across all utilized concentrations.

These data clearly demonstrate the high resistance exhibited by these strains against green tea extracts, proving that the effect of tea is entirely weak against them at these current limits and concentrations.

This negative outcome is in agreement with the findings established by **(Natarajan *et al.* 2025)** , who also confirmed that green tea extracts are ineffective against the Aspergillus niger strain across all used concentrations, thereby validating our results regarding the low efficiency of these extracts against these types of fungi.

IV.3.3.3 Determination of MIC and MBC :

To evaluate the microbicidal and inhibitory potencies of our preparations, the minimum inhibitory concentrations (MIC) alongside the minimum bactericidal concentrations (MBC)

were assessed. This evaluation targeted four bacterial strains (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Bacillus cereus*) exposed to three distinct extract types (infusion, decoction, and ethanolic) derived from the green tea brands under study (El-Berrad and El-Raqim). The compiled quantitative data are organized in the tables below.

- Negative Control: Liquid growth medium (Mueller-Hinton broth) inoculated solely with the bacterial suspension.
- Positive Control: Bacterial suspension combined with the liquid growth medium and supplemented with a standard reference antibiotic.

Table 15: Minimum inhibitory concentrations (MIC) of the three tea extracts for both brands against the four bacterial strains

Samples.	Extracts.	The minimum inhibitory concentrations (mg/ml).			
		E. coli	*S. aureus*	*B. subtilis*	*B. cereus*
El-Berrad	**Infusion.**	17.5	2.18	8.75	17.5
	Aqueous extract.	8.75	1.09	8.75	17.5
	Ethanolic extract.	8.75	1.09	2.18	17.5
El - Rakim	**Infusion.**	17.5	2.18	8.75	17.5
	Aqueous extract.	8.75	2.18	4.37	17.5
	Ethanolic extract.	8.75	1.09	1.09	17.5

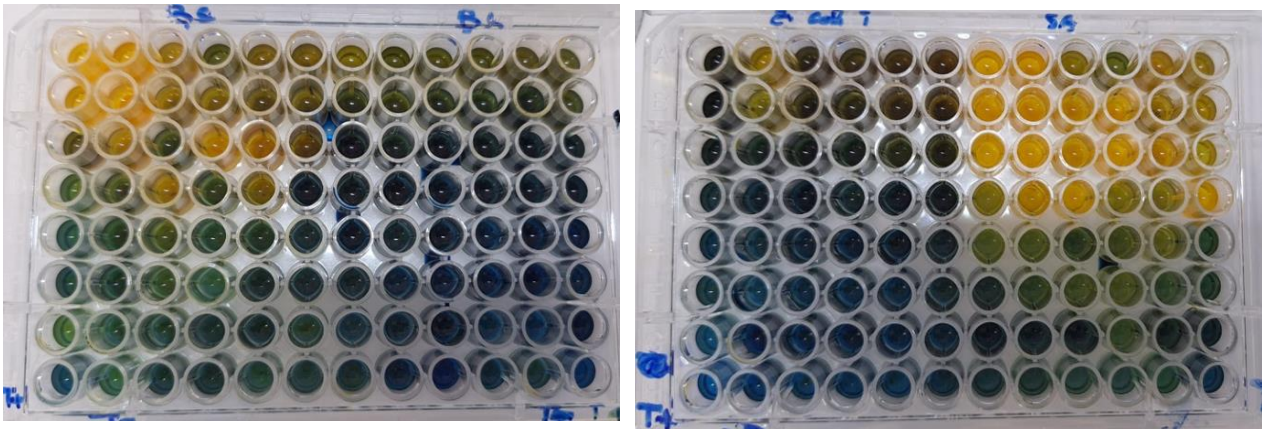


Figure 42: Results of MIC of the three tea extracts for both brands against the four bacterial strains.

The results in the table14 and figure 41 show the minimum inhibitory concentrations (MIC) of the three tea extracts against the four bacterial strains.

In general, we notice that the MIC values of all extracts are very close, meaning that no single extract showed a huge superiority over the others. Instead, the main difference lies in how each bacterial strain responded to these extracts.For Escherichia coli, the lowest MIC was 8.75 mg/mL for both the decoction and ethanolic extracts of both tea brands.

On the other hand, Staphylococcus aureus was much more sensitive; its lowest MIC was 1.09 mg/mL for the ethanolic and decoction extracts of El-Berrad, and for the ethanolic extract of El-Raqim.

Comparing these two strains, we can see that the extracts succeeded in inhibiting S. aureus at very low concentrations, while the concentration of 8.75 mg/mL for E. coli is considered high.For Bacillus subtilis, the lowest MIC was recorded for the ethanolic extracts, reaching 2.18mg/mL for El-Berrad and 1.09 mg/mL for El-Raqim. In contrast, for Bacillus cereus, all extracts stopped at a high concentration of 17.5 mg/mL This high value indicates that the extracts were not effective against this strain at lower concentrations.As a general comparison, Staphylococcus aureus was the most sensitive strain controlled by the extracts, with a maximum MIC of only 2.18 mg/mL.

Meanwhile, Bacillus cereus showed the highest resistance, with the lowest MIC against it being 17.5 mg/mL. Between the two tea brands, the results were very close. Finally, although the results are similar, the ethanolic extract slightly outperformed the others because it scored the lowest concentrations across almost all strains, except for B. cereus where all extracts were equal.

IV.3.3.4 Minimum Bactericidal Concentration (MBC) :

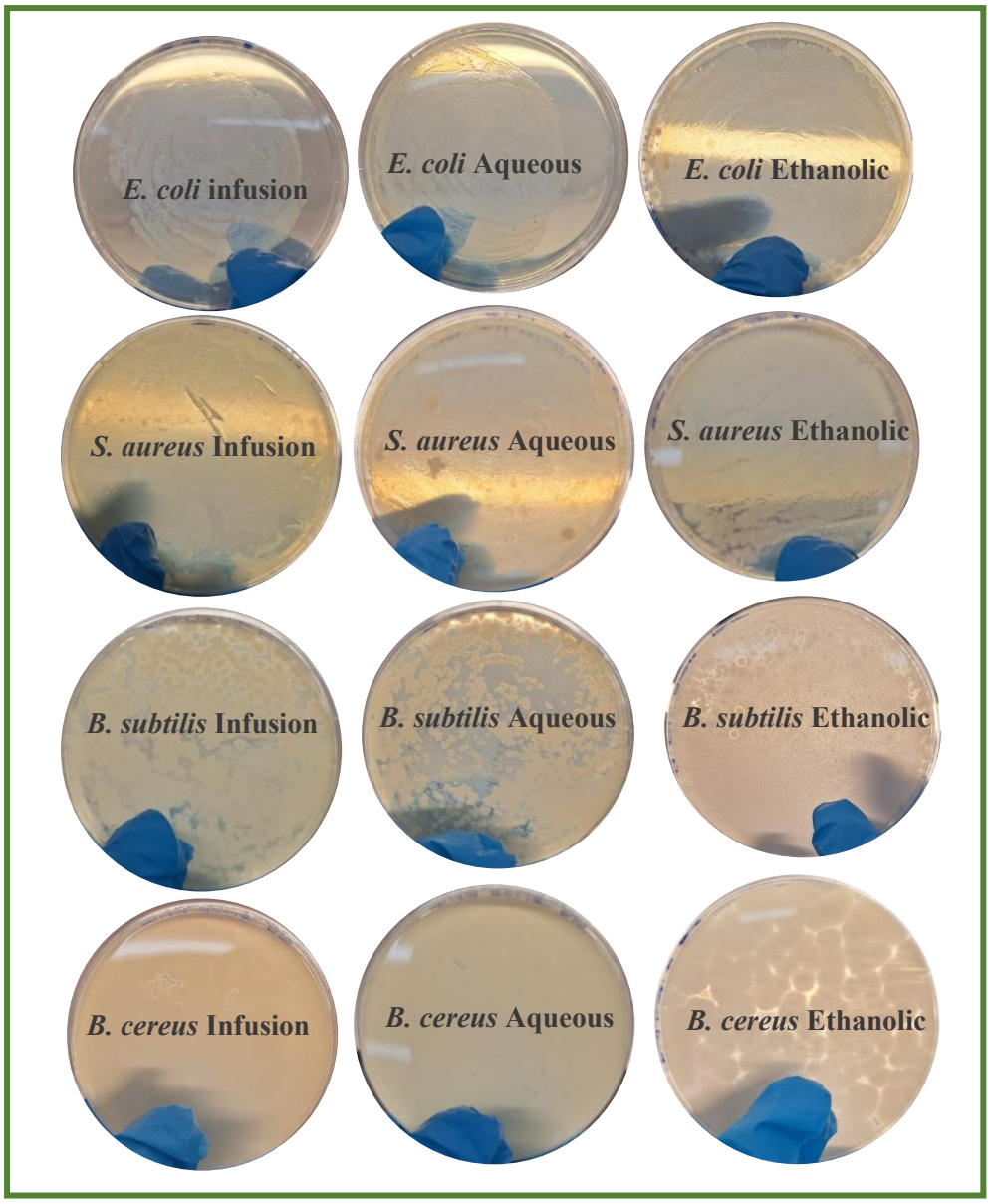


Figure 43: Results of MBC of the three tea extracts for both brands against the four bacterial strains.

The results of the current study demonstrate that the green tea extracts of the two samples, "El-Berrad" and "ElRakim", exhibit a bacteriostatic effect rather than a bactericidal one against the tested bacteria at the evaluated concentrations. This biological behavior can be attributed to the nature of the bioactive compounds present in green tea, primarily catechins and polyphenols.

Mechanistically, according to Taylor et al. (2005), natural catechins (such as ECg and EGCg) possess moderate antibacterial activity. Instead of aggressively attacking the bacteria and causing direct cell wall lysis, these molecules act through a more precise and selective

mechanism by binding to the cytoplasmic membrane and inducing profound alterations in its physical structure. This membrane perturbation leads to an increase in cell wall thickness and compromises the activity of cell separation enzymes (autolysins). Consequently, this disruption paralyzes the metabolic functions of the bacteria and halts their ability to replicate and divide without causing immediate cell death, a phenomenon scientifically defined as a bacteriostatic effect.

In conclusion, this discussion highlights the significant bioactive potential of the "El-Berrad" and "Er-Rakim" green tea brands from the Saïda region. The results indicate that the extraction of secondary metabolites is highly dependent on the solvent and thermal treatment, where ethanolic decoction optimized both antioxidant and anti-inflammatory activities. Mechanistically, antimicrobial testing revealed a bacteriostatic rather than a bactericidal effect against the bacterial strains, with no activity observed against fungi. Ultimately, the high chemical and biological convergence between "El-Berrad" and "Er-Rakim" demonstrates their full equivalence in quality and efficacy for the local consumer.

Conclusion

Medical plants remain a cornerstone in the advancement of the pharmaceutical sector, drawing significant scientific interest due to their therapeutic and protective secondary metabolites, such as flavonoids and catechins. Among these, Camellia sinensis (green tea) stands out as one of the most consumed beverages worldwide. It is deeply embedded in numerous cultures, particularly in Algeria, where it represents a traditional daily ritual. Driven by this cultural and medicinal importance, this study aimed to evaluate the biological activities and efficiency of this traditional beverage by selecting the two most consumed commercial brands in the Saïda region.

To mimic traditional preparation methods, the targeted bioactive compounds were extracted using two approaches: infusion and hot reflux, employing only water and ethanol as solvents. The preliminary phytochemical screening confirmed the presence of the investigated metabolites. Subsequently, the antioxidant capacity was evaluated using two complementary assays (DPPH and FRAP), revealing that green tea exhibits robust efficiency in scavenging free radicals. Furthermore, the anti-inflammatory activity was assessed via the red blood cell (RBC) membrane stabilization test, yielding unexpected and remarkable results; both extracts demonstrated excellent protective effects on RBC membranes, strongly validating the plant's anti-inflammatory potential. Conversely, the antimicrobial screening, performed using the agar well diffusion and minimum inhibitory concentration (MIC) methods, showed weak to moderate activity. It was noted that the extracts exert a dose-dependent effect, requiring higher concentrations to inhibit microbial growth, while exhibiting a strictly bacteriostatic rather than bactericidal action.

Throughout all biological assays, the ethanolic extract consistently demonstrated superior performance. Interestingly, the aqueous extract under reflux which reflects the traditional boiling preparation method utilized in some southern Algerian regions also showed notable efficacy. This observation suggests a practical recommendation to transition from the common simple infusion technique to the boiling method to achieve maximum recovery of bioactive compounds.

Regrettably, due to the unavailability of certain specific reagents, the quantitative determination of total phenols and flavonoids could not be performed, despite its critical importance in assessing plant quality. Additionally, time constraints prevented the testing of a broader series of concentrations in the anti-inflammatory assay to calculate the IC_{50} value.

Consequently, several perspectives are proposed for future cohorts:

- First, executing the quantitative analysis of phenolic compounds and flavonoids to precisely correlate them with the observed activities.

- Transitioning from in vitro laboratory screening to in vivo biological studies to evaluate the real therapeutic efficacy and safety of both green tea brands inside living organisms.
- Advanced Phytochemical Profiling: Performing a rigorous and precise identification of bioactive compounds using advanced chromatographic techniques, specifically HPLC (High-Performance Liquid Chromatography) .
- Investigating the bioavailability and metabolic pathways of green tea catechins (especially EGCG) to understand how they are absorbed and utilized biologically.
- Conducting dose-response studies based on literature parameters to determine the exact optimal concentrations required for maximum antimicrobial and anti-inflammatory effects.

Given that this plant is readily available in our markets and consumed daily, uncovering such astonishing therapeutic properties represents a valuable asset and a potential qualitative leap for both the functional food industry and pharmaceutical development.

Bibliographic References.

Ahmed, S., Alsharif, K. F., Aschner, M., Alzahrani, K. J., Akkol, E. K., Türkcanoğlu, E. G., & Khan, H. (2026). A deep dive into herbal extraction: Techniques, trends, and technological advancements. South African Journal of Botany, 188, 9–37. https://doi.org/10.1016/j.sajb.2025.11.005.

Aidoo DB, Konja D, Henneh IT, Ekor M. Protective Effect of Bergapten against Human Erythrocyte Hemolysis and Protein Denaturation in Vitro. Int J Inflam 2021;2021.

Ali, B., & Sharma, S. N. (1968). Interaction of some non-steroidal anti-inflammatory drugs with serum proteins. Journal of Pharmacy and Pharmacology, 20(3), 169–173. https://doi.org/10.1111/j.2042-7158.1968.tb09719.

Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). DARU, Journal of Pharmaceutical Sciences 2012;20. https://doi.org/10.1186/2008-2231-20-76PMid:23351977.

Arya, P., Dabra, A., & Guarve, K. (2019). Green tea: Chemical composition, biological effects and health benefits. Asian Journal of Pharmacy and Pharmacology, 5(2), 227-234. https://doi.org/10.21102/ajpp.2019.050202.

Azab, A., Nassar, A., & Azab, A. N. (2016). Anti-inflammatory activity of natural products. Molecules, 21(10), 1321. https://doi.org/10.3390/molecules21101321.

Benfedda, D., & Ouaret, A. (2025). Evaluation de l'effet antioxydant et antidépresseur de Camellia sinensis [Mémoire de Master en Toxicologie]. Université Constantine 1 Frères Mentouri, Algérie.

Book of Tea: Cultivation to consumption Edited by **K. C. Willson and M. N.** Clifford © 1992 Chapman & Hall, London. ISBN 0412338505.

Bouandoura, N., & Bendimerad, N. (2012*).* Evaluation of the antioxidant activity of aqueous and methanolic extracts of Satureja calamintha ssp. Nepeta (L.) Briq. Nature & Technology, (07), 10-14.

Boudou F, Belakredar A, Keziz A, Alsaeedi H, Cornu D, Bechelany M and Barhoum A (2025) Camellia sinensis phytochemical profiling, drug-likeness, and antibacterial activity

against gram-positive and gram-negative bacteria: in vitro and in silico insights. Front. Chem. 13:1555574.

BOUSSAYED, M., & REMMAS, A. (2025). Évaluation de l'activité antioxydante et antimicrobienne de la pelure de grenade de la région de Saïda (Mémoire de Master en Biologie). Université de Saïda - Dr Moulay Tahar, Algérie.

Brimson, J. M., Prasanth, M. I., Kumaree, K. K., Thitilertdecha, P., Malar, D. S., Tencomnao, T., & Prasansuklab, A. (2023). Tea plant (Camellia sinensis): A current update on use in diabetes, obesity, and cardiovascular disease. Nutrients, 15(1), 37. https://doi.org/10.3390/nu15010037.

Candela, L., Formato, M., Crescente, G., Piccolella, S., & Pacifico, S. (2020). Coumaroyl flavonol glycosides and more in marketed green teas: An intrinsic value beyond much-lauded catechins. Molecules, 25(8), 1765. https://doi.org/10.3390/molecules25081765.

Chen, Q., Zhu, Y., Dai, W., Lv, H., Mu, B., Li, P., Tan, J., Ni, D., & Lin, Z. (2019). Aroma formation and dynamic changes during white tea processing. Food Chemistry, 274, 915–924. https://doi.org/10.1016/j.foodchem.2018.09.072

Cooper, R., Morré, D. J., & Morré, D. M. (2005). Medicinal benefits of green tea: Part I. Review of noncancer health benefits. The Journal of Alternative and Complementary Medicine, 11(3), 521–528. https://doi.org/10.1089/acm.2005.11.521

Das N. Kalita K. Boruah P .Sarma U. (2017). Prediction of moisture loss in withering process of tea manufacturing using artificial neural network. IEEE Trans. Instrum. Meas.67, 175–184. doi: 10.1109/TIM.2017.2754818

doi: 10.1038/s41438-021-00542-x.

Flieger J , Flieger W , Baj J , Maciejewski R . Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. Materials. 2021;14(15):4135. Available from: https://doi.org/10.3390/ma14154135.

Fujiati, F., Haryati, H., Joharman, J., & Utami, S. W. (2022). In vitro metabolite profiling and anti-inflammatory activities of Rhodomyrtus tomentosa with red blood cell membrane

stabilization methods. Research in Molecular Medicine, 11(3), 502-511. https://doi.org/10.52547/rbmb.11.3.502.

Gorla, U. S., Savithri, M., Rao, G. S. N. K., Niharika, Y., Sathya, P. D. S., & Harika, V. (2018). Evaluation of anti-inflammatory activity of hydroalcoholic extract of Ananas comosus fruit peel by HRBC membrane stabilisation. Asian Journal of Pharmaceutical Research, 8(1), 33-35. https://doi.org/10.5958/2231-5691.2018.00007.X.

Goudja, A. M., Koularambaye, K., Guedembaye, G., & Maoundombaye, T. (2022). Ethnobotanical study and phytochemical screening of medicinal plants used in the treatment of hemorrhoids in three provinces of South-Chad. International Journal of Biological and Chemical Sciences, 16(5), 2132-2147.

H'maimed, M. A. (2016). Étude phytochimique et activité biologique des composés phénoliques des deux plantes médicinales du sud algérien (Juniperus phoenicea L., et Cotula cinerea (Del).) (Thèse de Doctorat/Mémoire de Magister). Université Dr. Moulay Tahar, Saïda, Algérie.

Hayat, K., Iqbal, H., Malik, U., Bilal, U., & Mushtaq, S. (2015). Tea and its consumption: Benefits and risks. Critical Reviews in Food Science and Nutrition, 55(7), 939–954. https://doi.org/10.1080/10408398.2012.678949.

Hojo, A. (2017). Introduction of various types of tea trees and their characteristics. HOJO Tea. Retrieved June 1, 2026, from https://hojotea.com/.

Kaddouri, M. (2024). Criblage phytochimique et bioactivité (antifongique, antioxydant) des extraits de deux plantes (Artemisia herba-alba et Allium sativum) de la wilaya de Saïda (Mémoire de Master en Biologie). Université Dr. Moulay Tahar, Saïda, Algérie.

Karumi, Y. (2004). Phytochemical screening and biological activities of plant extracts [Unpublished manuscript/Thesis]. Department of Biochemistry, College of Medical Science, University of Maiduguri, Nigeria.

Koch, W., Kukula-Koch, W., Komsta, Ł., Marzec, Z., Szwerc, W., & Głowniak, K. (2018). Green tea quality evaluation based on its catechins and metals composition in combination with chemometric analysis. Molecules, 23(7), 1689. https://doi.org/10.3390/molecules23071689.

Lü, J. M., Lin, P. H., Yao, Q., & Chen, C. (2010). Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. *Journal of Cellular and Molecular Medicine*, *14*(4), 840-860. https://doi.org/10.1111/j.1582-4934.2009.00897.

Mansoori, R., Jain, D., Pandey, V., & Jain, S. K. (2022). A comprehensive review on biological activity of green tea (Camellia sinensis). Journal of Drug Delivery and Therapeutics, 12(5), 250-265. https://doi.org/10.22270/jddt.v12i5.5590.

Martin, P., Malenga, N., Mphangwe, N., Nyirenda, H., & Rattan, P. (1997). A review of current recommendations for pruning tea. Quarterly Newsletter - Tea Research Foundation of Central Africa, 126, 21–33.

Mehrabani, M., Amirheidari, B., Ahmadinia, N., Forooghi-Nematollahi, M., & Sabouri, S. (2022). Antioxidant and cytotoxic effects of aqueous extract of Iranian green tea (Camellia sinensis L.). Iranian Journal of Pharmaceutical Sciences, 18(1), 77-86. https://doi.org/10.22034/ijps.2021.529851.1788.

Natarajan, K., Thayumanavan, T., & Subashkumar, R. (2025). Antioxidant, Antibacterial and Antifungal Activity of Camellia sinensis. Research Journal of Pharmacy and Technology, 18(6), 2475-2479. https://doi.org/10.52711/0974-360X.2025.00353

Nazliniwaty, N., Hanum, T. I., & Laila, L. (2020). Antioxidant activity test of green tea (Camellia sinensis L. Kuntze) ethanolic extract using DPPH method. In Proceedings of the International Conference of Science, Technology, Engineering, Environmental and Ramification Researches (ICOSTEERR 2018) (pp. 752-754). SCITEPRESS. https://doi.org/10.5220/0010087307520754.

Nurtamin, T., Rahim, A. H., & Yusuf, M. (2018). Evaluation of anti-inflammatory activity by human red blood cell membrane stabilization method. Asian Journal of Pharmaceutical and Clinical Research, 11(5), 176–179. https://doi.org/10.22159/ajpcr.2018.v11i5.24430

Nwachukwu, I. D., Sarteshnizi, R. A., Udenigwe, C. C., & Aluko, R. E. (2021). A concise review of current in vitro chemical and cell-based antioxidant assay methods. Molecules, 26(16), 4865. https://doi.org/10.3390/molecules26164865

Omran, A. M., Al Sa'ady, A. T., & Saleh, R. H. (2023). Phytochemical screening, antibacterial activity, and chromatographic study of Camellia sinensis. Medical Journal of Babylon, 20(4), 790-796. https://doi.org/10.4103/MJBL.MJBL_308_23.

Pacheco-Coello, F., & Franco-De Jesús, L. (2021). Evaluation of the anti-inflammatory activity of aqueous and methanolic extracts of Hibiscus sabdariffa and Camellia sinensis. Journal Name.

Parmar, N., Rawat, M., & Kumar, J. V. (2012). Camellia sinensis (Green tea): A review. International Journal of Pharmaceutical Sciences and Research, 3(2), 52-59.

Pathak, D. P., Bhutani, R., Kapoor, G., Kant, R., & Agarwal, U. (2017). Review on Camellia sinensis: Nature's gift. Journal of Pharmacognosy and Phytochemistry, 6(4), 11-14.

Peiris ,DSHS, DTK Fernando, SPNN Senadeera, and CB Ranaweera. 2025. "Assessment of In Vitro Anti-Inflammatory Activity: A Comprehensive Review of Methods, Advantages, and Limitations". Asian Journal of Research in Biochemistry 15 (2):37-52. https://doi.org/10.9734/ajrb/2025/v15i2365.

Planted Well. (2022, November 9). 37 Different types of tea and their benefits explained. Retrieved June 1, 2026, from https://plantedwell.com/types-of-tea/

POWO. (2024). Camellia sinensis (L.) Kuntze. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Retrieved June 1, 2026, from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:828549-

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (Camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. Nutrients, 11(2), 474. https://doi.org/10.3390/nu110204747.

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (Camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. Nutrients, 11(2), 474. Department of Clinical Chemistry, Chulalongkorn University, Bangkok, Thailand & Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. https://doi.org/10.3390/nu11020474.

Radeva-Ilieva, M., Stoeva, S., Hvarchanova, N., & Georgiev, K. D. (2025). Green tea: Current knowledge and issues. Foods, 14(5), 745. https://doi.org/10.3390/foods14050745.

Ramírez-Aristizabal, L. S., Ortíz, A., Restrepo-Aristizabal, M. F., & Salinas-Villada, J. F. (2017). Comparative study of the antioxidant capacity in green tea by extraction at different temperatures of four brands sold in Colombia. Vitae, Revista de la Facultad de Ciencias

Farmacéuticas y Alimentarias, 24(2), 132-145. http://dx.doi.org/10.17533/udea.vitae.v24n1a06.

Reto, M., Figueira, M. E., Filipe, H. M., & Almeida, C. M. M. (2007). Chemical composition of green tea (Camellia sinensis) infusions commercialized in Portugal. Plant Foods for Human Nutrition, 62(4), 139–144. https://doi.org/10.1007/s11130-007-0054-3

Rietveld, A., & Wiseman, S. (2003). Antioxidant effects of tea: Evidence from human clinical trials. The Journal of Nutrition, 133(10), 3285S–3292S. https://doi.org/10.1093/jn/133.10.3285S.

Schoorel, A. F., & van der Vossen, H. A. M. (2000). Camellia sinensis (L.) Kuntze. In H. A. M. van der Vossen & M. Wessel (Eds.), Plant Resources of South-East Asia No 16: Stimulants (pp. 57-64). PROSEA Foundation.

Sharma, N., Deshpande, S., Ganjoo, N., & Aman, A. (2022). Botanical description, phytochemistry, traditional uses, and pharmacology of green tea (Camellia sinensis): An updated review. International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS), 11(1, Special Issue 2), 76-88. https://doi.org/10.31032/IJBPAS/2022/11.1.2008

Shon, M.Y.; Choi, S.D. ; Kohng, G.G. ; Nam, S.H ; Sung, N.J (2004) . Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onion. Food Chem. Toxicol., 42, 659-666.

Shrivastava, R. R., Pateriya, P., & Singh, M. (2018). Green tea: A short review. Journal of Drug Delivery and Therapeutics, 8(6), 415-420.

Singh, A., Patidar, D., Gupta, N., & Rajput, D. S. (2023). Physiochemical investigation and evaluation of Camellia sinensis leaves for in vitro antioxidant and anti-inflammatory activity. International Journal of Pharmaceutical Sciences and Research, 14(8), 3945-3951.

Subramanian, N., Venkatesh, P., Ganguli, S., & Sinkar, V. P. (1999). Role of polyphenol oxidase and peroxidase in the generation of black tea theaflavins. Journal of Agricultural and Food Chemistry, 47(7), 2571–2578. https://doi.org/10.1021/jf981042y

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14(6), 2167-2180.

Tang, G. Y., Meng, X., Gan, R. Y., Zhao, C. N., Liu, Q., Feng, Y. B., Li, S., Wei, X. L., Atanasov, A. G., Corke, H., & Li, H. B. (2019). Health functions and related molecular mechanisms of tea components: An update review. International Journal of Molecular Sciences, 20(24), 6196. https://doi.org/10.3390/ijms20246196

Tanim, J. H. (2024). Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. European Journal of Microbiology and Immunology, 14(2), 97–115. https://doi.org/10.1556/1886.2024.00018.

Tariq, A. L., Nirjantha, D., & Reyaz, A. L. (2013). Antimicrobial activity of Camellia sinensis leaves against Gram-positive and Gram-negative bacteria. 2013-2012. Retrieved from ResearchGate.

Taylor, P. W., Hamilton-Miller, J. M. T., & Stapleton, P. D. (2005). Antimicrobial properties of green tea catechins. Food Science and Technology Bulletin, 2, 71–81.

Tian, J., Geiss, C., Zarse, K., Madreiter-Sokolowski, C. T., & Ristow, M. (2021). Green tea catechins EGCG and ECG enhance the fitness and lifespan of Caenorhabditis elegans by complex I inhibition. Aging, 13(19), 22679–22696. https://doi.org/10.18632/aging.203611.

Truong, V. L., & Jeong, W. S. (2021). Cellular defensive mechanisms of tea polyphenols: Structure-activity relationship. International Journal of Molecular Sciences, 22(17), 9109. https://doi.org/10.3390/ijms221791095.

Wakamatsu, M., Yamanouchi, H., Sahara, H., Iwanaga, T., Kuroda, R., Yamamoto, A., Minami, Y., Sekijima, M., Yamada, K., & Kajiya, K. (2019). Catechin and caffeine contents in green tea at different harvest periods and their metabolism in miniature swine. Food Science & Nutrition, 7(8), 2769–2778. https://doi.org/10.1002/fsn3.1137.

Walsh, J. M. (1892). Tea: Its history and mystery (Illustrated ed.). Coates.

Wang P. Yu J. Jin S. Chen S. Yue C .Wang W.. (2021). Genetic basis of high aroma and stress tolerance in the oolong tea cultivar genome. Hortic. Res.8:107.

Wang, C., Han, J., Pu, Y., & Wang, X. (2022). Tea (Camellia sinensis): A review of nutritional composition, potential applications, and omics research. Applied Sciences, 12(12), 5874. https://doi.org/10.3390/app12125874

Wikifarmer. (2023). Tea plant complete cultivation guide. Wikifarmer Library.

Wong M. Sirisena S. Ng K. (2022). Phytochemical profile of differently processed tea: a review. J. Food Sci.87, 1925–1942. doi: 10.1111/1750-3841.16137.

Wong, M., Sirisena, S., & Ng, K. (2022). Phytochemical profile of differently processed tea: A review. Journal of Food Science, 87(5), 1925–1942. https://doi.org/10.1111/1750-3841.16135.

Xing, L., Zhang, H., Qi, R., Tsao, R., & Mine, Y. (2019). Recent advances in the understanding of the health benefits and molecular mechanisms associated with green tea polyphenols. Journal of Agricultural and Food Chemistry, 67(4), 1029–1043. https://doi.org/10.1021/acs.jafc.8b06146

YeJ.- H .Ye Y. Yin J.-F .Jin J .Liang Y.- R. Liu R .-Y. (2022). Bitterness and astringency of tea leaves and products: formation mechanism and reducing strategies. Trends Food Sci. Tech.123, 130–143. doi: 10.1016/j.tifs.2022.02.031.

Yoshan Tea. (2023, April 7). Is the tea with one bud and two leaves better to drink? Understanding the flavor of tea leaves.

Zhao, T.; Li, C.; Wang, S.; Song, X(2022). Green Tea (Camellia sinensis): A Review of Its Phytochemistry, Pharmacology, and Toxicology. Molecules,(27), 3909.

Annexes

Annex :

Preparation of Reagents:

Wagner's Reagent: Dissolve 2 g of KI and 1.27 g of I_2 in 75 mL of water. Adjust the total volume to 100 mL with water.

Mayer's Reagent: Dissolve 1.358 g of $Hg Cl_2$ in 60 mL of water and separately dissolve 5 g of KI in 10 mL of water. Mix both solutions, then adjust the total volume to 100 mL with water.

Starch Reagent: Dissolve 1.2 g of iodine in 50 mL of distilled water containing 2.5 g of potassium iodide. Heat 5 mL of the test solution with 10 mL of a saturated NaCl solution in a water bath until boiling.

Preparation of the DPPH Solution:

The DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL of methanol. To ensure the stability of the DPPH radical, the solution was stored protected from light at a temperature of 20 °C.

The absorbance of the solution was then adjusted to 0.5 at 517 nm using a spectrophotometer.

Preparation of the K_3 Fe$(CN)_6$ Solution:

The potassium ferricyanure K_3 Fe$(CN)_6$ solution was prepared by dissolving 1 g of the compound in 100 mL of distilled water.

Preparation of the 10% Trichloroacetic Acid (TCA) Solution:

The 10% (w/v) TCA solution was obtained by dissolving 1 g of trichloroacetic acid in 10 mL of distilled water.

Preparation of the 0.1% Ferric Chloride ($FeCl_3$) Solution:

The 0.1% (w/v) ferric chloride ($FeCl_3$) solution was prepared by dissolving 0.1 g of ($FeCl_3$) in 100 mL of distilled water.

Preparation of PBS (Phosphate Buffered Saline) Buffer:

For the preparation of a 0.01 M PBS buffer at pH 6.4: The 0.01 M Phosphate Buffered Saline (PBS) solution at pH 6.4 was prepared by dissolving 1.44 g of disodium phosphate ($Na_2 HPO_4$), 0.24 g of monopotassium phosphate (KH_2PO_4), 8.0 g of sodium chloride (NaCl), and 0.2 g of

potassium chloride (KCl) in a volume of distilled water, which was then adjusted to a final volume of 1 L.

Preparation of Alsever's Solution:

Alsever's solution was prepared by dissolving 2 g of dextrose (2% w/v), 0.8 g of sodium citrate (0.8% w/v), 0.5 g of citric acid (0.5% w/v), and 0.42 g of sodium chloride (NaCl, 0.42% w/v) in a volume of distilled water, which was then adjusted to a final volume of 1 L.

Table 16: DPPH antioxidant activity results of the tea extracts for both brands, positive control.

Samples.	**Extracts.**	**Inhibition %.**				
		1.25 (mg/ml).	0.62 (mg/ml).	0.31 (mg/ml).	0.15 (mg/ml)	IC50 (mg/ml).
El-Berrad	**Infusion.**	94,58	73,05	51,64	40	0.28
	Aqueous extract.	84,82	66,58	56,47	47,41	0.16
	Ethanolic extract.	97	84,62	64,5	48,5	0.014
El - Rakim	**Infusion.**	88,25	72,4	56,75	40,87	0.18
	Aqueous extract.	83,75	74,62	57	45.75	0.12
	Ethanolic extract.	90,84	84.57	61,80	47,46	0.015
Ascorbic acid		99.43	99.32	99.09	85.00	0.00034

Table 17: FRAP antioxidant activity results of the tea extracts for both brands, positive control.

Samples	Abs / Extracts	1 (mg/ml).	0.5 (mg/ml).	0. 25 (mg/ml).	0. 125 (mg/ml)	0.062 (mg/ml).	0.031 (mg/ml).	0.001 (mg/ml).	0.007 (mg/ml).	0.003 (mg/ml).	0.001 (mg/ml).	EC50 (mg/ml) .
El-Berrad	**Infusion**.	/	1.197	0.754	0.276	0.188	0.12	0.117	/	/	/	0.18
	Aqueous extract.	/	2.065	0.939	0.523	0.273	0.247	0.227	/	/	/	0.1
	Ethanolic extract.	/	1.78	1.146	0.555	0.178	0.148	0.127	/	/	/	0.1
El - Rakim	**Infusion**.	0,939	0,585	0,418	0,26	0,242	0,218	0,211	0,201	0,176	/	0.40
	Aqueous extract.	1.44	0.927	0.618	0.541	0.322	0.234	0.210	0.201	0.207	/	0.20
	Ethanolic extract.	/	2,334	1,326	1,037	0,631	0,448	0,417	0,391	0,295	0.287	0.036
Ascorbic acid		/	/	1.851	1.733	1.564	1.197	0.756	0.509	0.340	/	0.00081

Figure 44: UV-Vis spectrophotometer used for quantitative assays.