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THEME

Phytochemical study, biological evaluation and Ag Silver nanoparticle synthesis using *Psidium guajava* leaf extracts.

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الاهداء

بسم الله الرحمن الرحيم

(وآخر دعواهم ان الحمد لله رب العالمين)

صدق الله العظيم

الحمد لله الذي بفضله تتحقق الغايات من بعد الاستعانة به، وانهاء الدرب بتوفيقه وتحقق الحلم بفضله

إلى من قال الله تعالى فيهما:

(وقل رب ارحمهما كما ربياني صغيرا)

إلى العزيز الذي حملت اسمه فخرا، من بذل جهد السنين من اجل ان اعتلي سلالم النجاح، الى من غرس القوة بداخلي لأبحر في هذه الحياة، الى من علمني ان الدنيا كفاح وسلاحها العلم والمعرفة " والدي الحبيب"

إلى من علمتني الأخلاق قبل الحروف، إلى من كانت الداعمة الأولى، إلى من احتضنني قلبها قبل يدها، إلى التي ظلت دعواتها تضم اسمي وسهلت لي الشدائد بدعائها، إلى القلب الذي يبقيني حيا "والدتي الحبيبة"

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أماني

الإهداء

"و آخر دعواهم أن الحمد لله رب العالمين"

الحمد لله عند البدء و عند الختام

من قال أنا لها نالها

لقد كانت طريقا طويلة مليئة بالاخفاقات و النجاحات فخورين بكفاحنا لتحقيق احلامنا

لحظة لطالما انتظرتها و حلمت بها في حكاية اكتملت فصولها

الى فقيد قلبي، أبي الغالي...يا من غرست فيني حب العلم والطموح. كم تمنيت لو كنت هنا لتشاركني فرحتي، هذا الإنجاز هو نور أهديه لذكراك العطرة، ووعد مني بأن أسير على خطاك وأرفع اسمك عاليًا.

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الى كل هؤلاء اهدي هدا العمل وفقني الله و إياكم الى الخير.

إيمان

شكر وتقدير

لا يسعني إلا أن نعبر عن عميق امتناننا وشكرنا لأساتذتنا الذين لم يدخروا جهدًا في تقديم العون والتوجيه، وكانوا لنا بمثابة قدوة وملهمين.

كما نقدم عظيم الشكر لأعضاء لجنة المناقشة الموقرة، على قبولهم مناقشة هذا العمل، وعلى الرؤى الثاقبة والأسئلة الهامة التي طرحوها، والتي أثارت فهمنا للموضوع بشكل كبير. لكم منا كل التقدير

وأيضا وفاءً وتقديرًا واعترافا منا بالجميل نتقدم بجزيل الشكر للأستاذ المخلص الذي لم يألوا جاهدًا في مساعدتنا وتوجيهنا الدكتور "محمد العيد تليلي "

Abstract

Our study was based on a biological examination of extracts of the leaves of *Psidium guava*, a member of the Myceliaceae family, harvested from the Guemar region of Oued Souf province. The study sample was prepared, and the methanolic extract, essential oil, and silver nanoparticles (AgNPs) were obtained. The amount of polyphenols was estimated at (17.889 ± 0.167 mg gallic acid equivalent/g) and flavonoids at (0.313 ± 0.001 mg quercetin equivalent). Based on the results of evaluating the antioxidant activity of the extracts using the DPPH and FRAP assays, the results showed that the IC50 value for the methanolic extract was estimated at (0.984 ± 0.023 mg/ml), the IC50 value for the essential oil was estimated at (3.571 ± 0.179 mg/ml), and the IC50 value for the nanoparticles was estimated at (18.891 ± 1.4 mg/ml).

We then conducted an experimental study on a rat model to test the extracts' ability to treat burns. Polyphenols, alkaloids, and essential oil were extracted from *Psidium guava* leaves using appropriate extraction techniques, while silver nanoparticles (AgNPs) were prepared from the polyphenol extract. A 1cm diameter burn was inflicted on the dorsal surface of mice. The extracts were then applied at different concentrations topically to the burn in the rats model, compared with a positive control group (Mebo ointment was used) and a negative control group (burns were inflicted and left untreated). The efficacy of the extracts was evaluated over 12 days by measuring the burn shrinkage rate. The results showed that all *Psidium guava* leaf extracts (polyphenols, alkaloids, essential oil, and silver nanoparticles (AgNPs)) exhibited significant activity in promoting burn healing.

Keywords: *Psidium guava*, polyphenols, alkaloids, essential oil, AgNPS, burns**.**

Résumé

Notre étude s'est basée sur l'analyse biologique d'extraits de feuilles de goyave Psidium, un membre de la famille des Myceliaceae, récoltées dans la région de Guemar, province d'Oued Souf. L'échantillon d'étude a été préparé et l'extrait méthanolique, l'huile essentielle et les nanoparticules d'argent (AgNPs) ont été obtenus. La quantité de polyphénols a été estimée à (17,889 ± 0,167 mg équivalent acide gallique/g) et de flavonoïdes à (0,313 ± 0,001 mg équivalent quercétine). Sur la base des résultats de l'évaluation de l'activité antioxydante des extraits à l'aide des tests DPPH et FRAP, les résultats ont montré que la valeur IC50 pour l'extrait méthanolique a été estimée à (0,984 ± 0,023 mg/ml), la valeur IC50 pour l'huile essentielle a été estimée à (3,571 ± 0,179 mg/ml) et la valeur IC50 pour les nanoparticules a été estimée à (18,891 ± 1,4 mg/ml).

Nous avons ensuite mené une étude expérimentale sur un modèle de rat afin de tester l'efficacité des extraits pour traiter les brûlures. Des polyphénols, des alcaloïdes et de l'huile essentielle ont été extraits de feuilles de Psidium goyave à l'aide de techniques d'extraction appropriées, tandis que des nanoparticules d'argent (AgNPs) ont été préparées à partir de l'extrait de polyphénols. Une brûlure de 1 cm de diamètre a été infligée sur la face dorsale des souris. Les extraits ont ensuite été appliqués à différentes concentrations par voie topique sur la brûlure chez le modèle de rat, en comparaison avec un groupe témoin positif (pommade Mebo utilisée) et un groupe témoin négatif (brûlures infligées et non traitées). L'efficacité des extraits a été évaluée sur 12 jours en mesurant le taux de rétraction de la brûlure. Les résultats ont montré que tous les extraits de feuilles de Psidium goyave (polyphénols, alcaloïdes, huile essentielle et nanoparticules d'argent(AgNPs)) présentaient une activité significative pour favoriser la cicatrisation des brûlures.

Mots clés : Psidium goyave, polyphénols, alcaloïdes, huile essentielle, AgNPS, brûlures.

الملخص

كانت دراستنا مبينة على دراسة بيولوجية لمستخلصات اوراق بسيديوم الجوافة من عائلة الآسيات التي تم حصادها من منطقة قمار بولاية وادي سوف.

تم تحضير عينة الدراسة و الحصول على المستخلص الميثانولي و الزيت العطري و الجزيئات النانوية الفضية (AgNPs)، مع تقدير كمية البوليفينولات التي تم تقديرها ب(17.889 ± 0.167 مل جرام مكافئ حمض الغاليك / جرام) و الفلافونويدات ب(0.313 ± 0.001 مل جرام مكافئ كيرسيتين) و بناء على نتائج تقييم النشاط المضاد للأكسدة للمستخلصات باستخدام اختبار DPPH و اختبار FRAP ، أظهرت النتائج أن قيمة IC50 للمستخلص الميثانولي كانت مقدرة ب(0.984 ± 0.023مجم / مل)، و قيمة IC 50 للزيت العطري كانت مقدرة ب(3.571 ± 0.179 مجم / مل) ، قيمة IC 50 للجزيئات النانوية الفضية كانت مقدرة ب(18.891 ± 1.4 مجم / مل).

ثم قمنا بدراسة تجريبية على نموذج الفئران من أجل اختبار قدرة المستخلصات في علاج الحروق. تم تحضير مستخلصات البوليفينول والالكالويد والزيت العطري من أوراق بسيديوم الجوافة باستخدام تقنيات استخلاص مناسبة، بينما تم تحضير الجزيئات النانوية الفضية (AgNPs) من مستخلص البوليفينول. تم اجراء الحرق علو مستوى ظهر الفئران بقطر 1cm بعد ذلك تم تطبيق المستخلصات بتركيزات مختلفة موضعيا على الحرق في نموذج الفئران، مقارنة مع مجموعة تحكم إيجابية (استعمل مرهم ميبو) ومجموعة تحكم سالبة (احداث الحرق وتركه دون معالجة). تم تقييم فعالية المستخلصات على مدى 12 يوما من خلال قياس معدل انكماش الحرق. أظهرت النتائج ان جميع مستخلصات أوراق بسيديوم الجوافة البوليفينول والالكالويد والزيت العطري والجزيئات النانوية الفضية (AgNPs) أظهرت نشاطا ملحوظا في تعزيز التئام الحرق.

الكلمات المفتاحية: *Psidium guava* ، البوليفينول، القلويدات، الزيت العطري، AgNPS، الحروق.

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List of abbreviations

Abs: Absorbance
DPPH: 2,2-diphenyl-1-picrylhydrazyl
IC50: Inhibitory concentration
FRAP: FerricIon Reducing Antioxidant Power
I%: Percentage of inhibition
UV: Ultra-violet
%: Percentage
Mg EAG/g MS: milligram equivalent of gallic acid per gram of dry matter.
Mg QER / g MS: milligram equivalent of quercetin per gram of dry matter.

Summary

Chapter 01: Guava (Psidium guajava L)

Chapter 01:(Guava) Psidium guajava L .

1. Myrtaceae family

The name of the ecologically significant angiosperm family, the Myrtaceae, comes from the shrub "Myrtus," which grows in North Africa and South America close to the Mediterranean. There are roughly 140 genera and The family is an intriguing study group for individuals interested in the evolution of a big taxonomic group because of its odd distribution between 3800 and 5650 species **(Thais 2019; Sisir 2021)**.

Medicinal and aromatic plants belonging to family Myrtaceae are endowed with valuable medicinal properties and are highly enriched sources of plants' bioactive volatiles **(Fatema *et al*., 2023).**

Ornamental plants like Leptospernum (Australian tea tree), Eucalyptus, Verticordia (feather blossoms), and Callistemon (bottlebrush) are examples of well-known genera in the Myrtaceae. Eucalyptus is one of the Myrtaceae's economically significant taxa. (timber, essential oils), Syzygium (cloves, jamun, rose apple), Melaleuca (timber), Psidium (guava), and Pimenta (allspice, pimento, bay rum) **(Sisir *et al*., 2021)**. These species contain several phytochemicals belonging to different chemical classes such as phenolic acids, flavonoids, phloroglucinols, terpenoids, condensed and non-condensed tannins. Several factors are responsible for the quantitative and qualitative differences in the chemical composition of Myrtaceae plants **(Fatema *et al*., 2023).**

2. Guava (*Psidium guajava* L.)

2.1. General

The Myrtaceae family includes the guava (*Psidium guajava*) tree (Figure 1), a highly special and traditional plant that is grown for its many nutritional and therapeutic uses. In tropical regions, guava has been cultivated and used as a significant fruit. regions such as South America, Bangladesh, Pakistan, Indonesia, and India (**Kumar *et al*., 2021).**

Guajava Psidium Many indigenous medical systems use linn, mostly to address conditions relating to the stomach and gastrointestinal tract. Some ethnomedical use include crushing the leaves and applying the liquids that emerge on cuts, wounds, boils, ulcers, skin, and Rheumatic areas and soft tissue infected sites **(Chaudhari *et al*., 2020).**

Figure 1: *Psidium guajava* **(Shirur *et al*.,2013)**

2.2. Morphological Characteristics

Guava trees range in height from 2 to 7 meters and are small trees or shrubs with numerous branches. The tree's thin, smooth bark is copper in color and sheds easily. When the upper surface is removed, the greenish skin beneath it becomes visible. The trunk reaches a diameter of 25 cm once reaching full maturity. The tree has quadrangular twigs that are bent downward. The evergreen, leathery, short-petioled leaves are arranged opposite one another. The leaves are 7–15 cm long and 3–5 cm broad, with an uneven form that ranges from oval to elliptical. The foliage are made up of parallel lines, or veins, that radiate outward from the center axis. Clusters of 2–5 cm in diameter flowers with 4–5 white petals and 250 stamens are carried on the leaf axils. According to botany, guavas belong to the genus Psidium and family Myrtle. Depending on the cultivar, the fruit is round, ovoid, or pear shaped, weighing between 50 and 200 g and measuring 5 to 10 cm across. The fruit's exocarp has a thin, pale-yellow surface with a touch of pink. The fruit's fleshy mesocarp is located just adjacent to the exocarp. The flesh stretches up to 3–12 mm and is granular, thick, white, yellowish, or dark pink in color. Juicy, acidic, and flavorful is the mesocarp. The center pulp, or endocarp, is located beyond the granular meat. The endocarp contains rocky, yellowish seeds and is juicy, with a little deeper hue. The pulp has 112–535 seeds and measures 6 mm in diameter. The Stone cells and parenchyma cells are the two types of cell wall tissues found in the berry's pulpy endocarp. Fruit's distinctively grainy feel is caused by stone cells, which are made of lignified woody material and are impervious to enzyme digestion. Guava fruit's fast metabolism and high respiration rate give it a shelf life of roughly three to five days at room temperature **(Bazila *et al*., 2021).**

2.3. Various parts of P. guajava L., chemical constituents, and pharmacological activities.

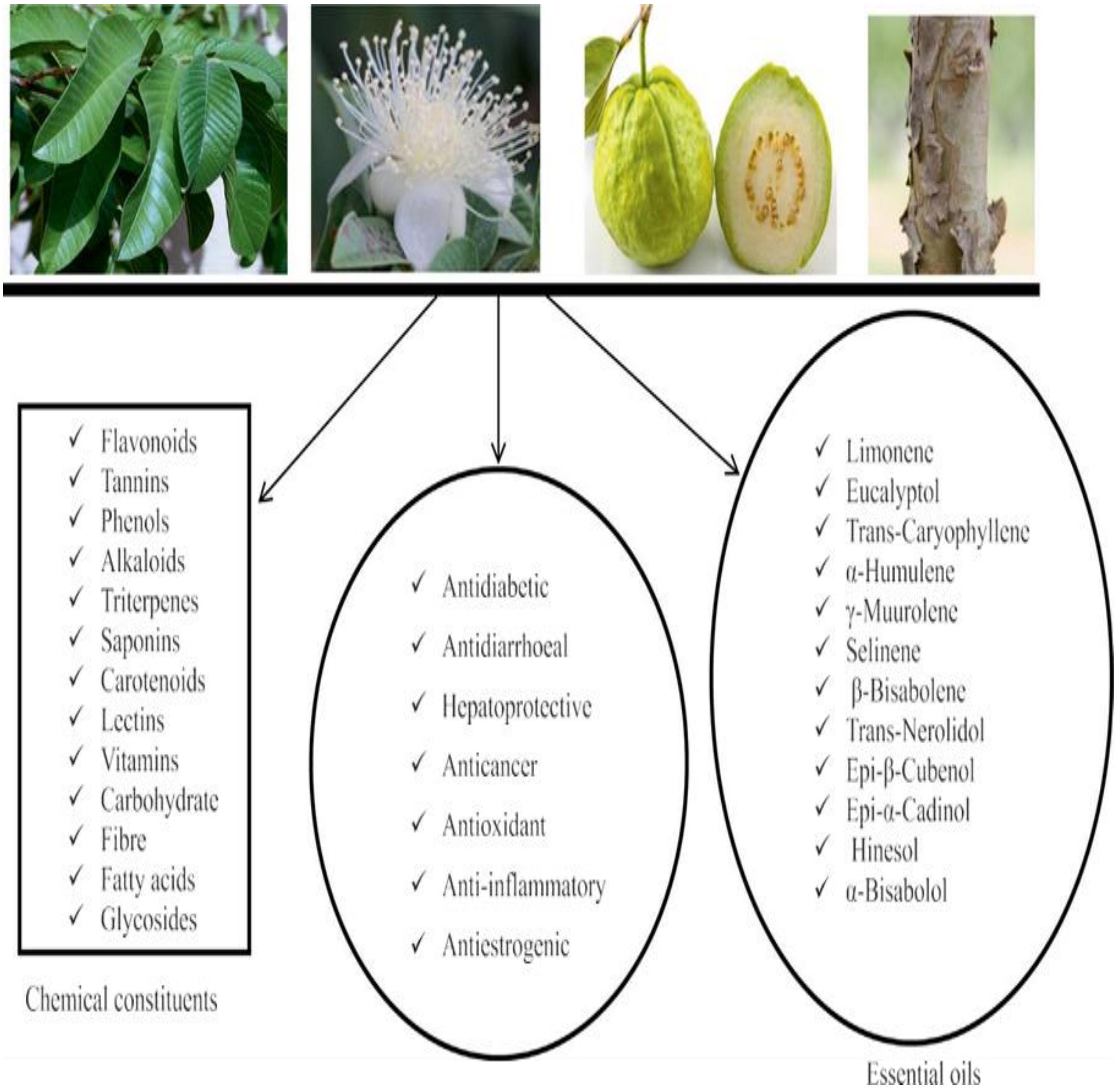

Figure2: Various parts of P. guajava L., chemical constituents, and pharmacological activities **(Eziuche *et al*.,2022).**

2.4. GEOGRAPHICAL SOURCE

Although guavas are thought to have originated in Mexico, they are also available in Asian nations due to their high nutritional content and health advantages. The nations that produce guavas are China, India, Thailand, Pakistan, and Mexico.

Nigeria, Bangladesh, Brazil, Indonesia, and the Philippines. India, Pakistan, and Brazil are the world's top producers of guava types **(Chaudhari *et al*.,2020).**

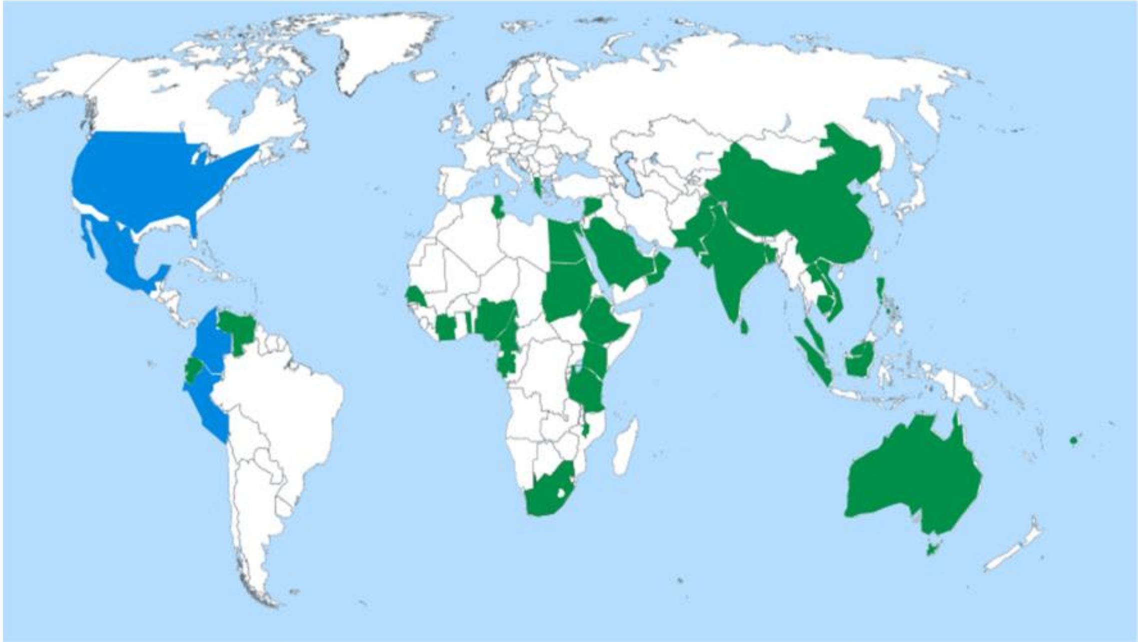

Figure 3: Geographical distribution of Pysidium guava **(Eziuche *et al*.,2022).**

Map of worldwide distribution of *Psidium guajava* ,the blue colour shows the native regions like Colombia, Mexico, Peru and United States of America, whereas the green colour represents the introduced countries such as Australia, Bangladesh, Brunei, Cambodia, Cameroon, China, Costa Rica, Cote d'Ivoire, Cuba, Dominican Republic, Ecuador, Eritrea, Ethiopia, Fiji, Gabon, Gambia, Greece, Guyana, Haiti, India, Indonesia, Kenya, Laos, Malawi, Malaysia, Myanmar, Nigeria, Pakistan, Panama, Philippines, Puerto Rico, Samoa, Senegal, South Africa, Sri Lanka, Tanzania, Thailand, Togo, Uganda, Venezuela, Vietnam, Egypt, Oman, Saudi Arabia, Palestine, Sudan, Syria and Tunisia **(Eziuche *et al*.,2022).**

2.5. The guava tree in Algeria

The French sent the guava tree to the botanical garden of the former agricultural school of Philippeville (Skikda) in Algeria in 1952. There was only one tree planted, and it is unknown what kind it is. In 2010, three more trees were successfully planted in the same plot. As an experiment, the guava tree was brought to central Algeria. Since 1978, a 2-hectare guava orchard at the level of a former communal agricultural estate (Das) has been established on a farm in the Fouka region of Tipaza, which is currently

the sole agricultural exploitation specialized in guava production with 350 trees. Some people believe that this fruit was brought to this area straight from the Middle East, while others believe that the colonists brought it back from Latin America. Given that certain colonial homes in Fouka usually had one or two guava trees in their gardens, this last theory is the most likely. Even the Jardin d'Essais du Hamma in Algiers had it planted **(BOUCHOUKH.,2021).**

2.6. International name

The following table shows the common names of the guava plant in some countries of the world.

Table 01: vernacular names

English	**guava**
French	goyavier, goyave
Spanish	guayaba, guayabo, guayaba manzana
German	Echte Guave
Indonesian	jambu batu, jambu biji
Italian	guaiava
Afrikaans	koejawel
Portuguese	Guaiaba, guaiava, goiabeira, goiabeiro, araça-goiaba, araça-guaçu

3. Taxonomy (BOUCHOUKH.,2021)

Domain	*Biota*
Kingdom	*Plantae*
Sub-kingdom	Viridaeplantae
Infra-Kingdom	Streptophyta
Class	Equisetopsida
Clade	Tracheophyta
Clade	Spermatophyta
Subclass	Magnoliidae
Super Order	Rosanae

Order	Myrtales
Family	Myrtaceae
Subfamily	Myrtoideae
Tribe	Myrteae
Subtribe	Psidiinae
Genus	Psidium
Species	*Psidium guajava*

4. Guava Varieties

The common guava is a diploid (2n = 22), but there are natural and artificial triploids (2n = 33), tetraploids (2n = 44; El Salvador species), hexaploids (2n = 66, Costa Rican species), and aneuploids. Triploids usually produce seedless fruits. Seedlings of hexaploids var. littorale are extremely uniform in plant and fruit characteristics. Seedling trees of most cultivated varieties vary in strength and size, fruit production and yield, shape, size, quality, season of ripening, and storage capacity. Around 100 different types of *Psidium guajava* are grown worldwide. Among the many varieties in this group are "Mexican Cream" and "Ruby X." "Hong Kong Pink." Lucknow 49 (Sardar), "Klom Toon," "White Indian," "Allahabad Safeda," "Red Fleshed," "Chittidar," "Nasik," and "Tathem White", The Supreme and Elizabeth. Certain varieties, such as "Pink Acid," "Patillo," and "Ka Hua Kula," produced highly acidic fruits with rosée or rouge pulp and a high pulp recovery percentage.Others produce fruits using a mixture of the two previously mentioned groups, such as Beaumont, Etherridge Selection, Oakey Pink, and Fanretief **(Bouchoukh., 2021).**

5. Chemical Composition

5.1 Chemical composition of guava fruit

Table02: Chemical composition of guava fruit **(Bazila *et al*.,2021)**

Nutrients	Per 100 g
Calories	68 kcal
Protein	2.55 g
Total Lipids	0.95 g
Carbohydrates	14.23 g
Moisture	88.1 g

Total dietary Fibre	2.4 g
Calorific value	285 kJ
Calcium	18 mg
Iron	0.26 mg
Sodium	2 mg
Potassium	417 mg
Zinc	0.23 mg
Selenium	0.6 mcg
Thiamine	0.067 mg
Riboflavin	0.04 mg
Niacin	1.084 mg
Iron	0.6 mg
Vitamin A	624 IU
Carotene beta	374 mcg
Vitamin C	228.3 mg
VitaminE alpha-tocopherol	0.73 mg
Vitamin K phylloquinone	2.6 mcg

5.2. Proximate Composition

Guava leaves (GLs) are abundant in micro- and macronutrients that promote health. trients and bioactive substances. In terms of total phenolic components, they are composed of 82.47% moisture, 3.64% ash, 0.62% fat, 18.53% protein, 12.74% carbs, 103 mg ascorbic acid, and 1717 mg gallic acid equivalents (GAE) (**Kumar *et al.*, 2021).**

5.3. Polysaccharides

In nature, polysaccharides are macromolecules that are widely distributed. They consist of lengthy chains of polymers made up of monosaccharide molecules. These polysaccharides exhibit a range of biological, pharmacological, and physicochemical characteristics, including immunomodulatory, anticancer, antidiabetic, anti-inflammatory, and antioxidant effects. About 9.13% uronic acid and 64.42% total sugar, of which 2.24% are reducing sugars, are included in these GLPs (**Kumar *et al.*, 2021).**

5.4. Proteins

On a dry weight basis, guava leaves contain 9.73% protein. Large macromolecules made up of amino acids, proteins serve as the building blocks of cells. Proteins are essential for cell signaling, enzyme regulation, growth and maintenance, and cited as biocatalysts16.8 mg protein per 100 g and 8 mg amino acids per 100 g According to Lowry's and Niinhydrin's techniques, respectively, uavaleaves are estimated. According to Jassaletal, because guavaleaves are a high source of proteins, carbs, and dietary fiber, they can be used as a sustainable and nutritious food source (**Kumar *et al*., 2021).**

5.5. Minerals and Vitamins

Minerals like calcium, potassium, sulfur, sodium, iron, boron, magnesium, manganese, and vitamins C and B are abundant in guava leaves. They are an excellent option for human nutrition and as animal feed to combat micronutrient deficiencies due to their greater concentrations of magnesium, sodium, sulphur, manganese, and bingle-globules.Thomasetal. found that the concentrations of minerals such calcium, phosphorus, potassium, iron, and magnesium were 1660, 360, 1602, 13.50, and 440 mg per 100 grams of dry weight (DW), respectively.Vitamins C and B had corresponding concentrations of 103.0 and 14.80 mg per 100 gDW.Eating GLs rich in calcium and phosphorus lowers the risk of disorders linked to deficiencies, including as osteoporosis, hypophosphatemia, and hypocalcemia.The concentration of calcium, phosphorus, magnesium, iron, and vitamin B in GLs was also higher than that of uavafruit, according to the study.While vitamin B is crucial for enhancing blood circulation, nerve relaxing, and cognitive function stimulation, the increased vitamin C concentration in GLs may aid in boosting the immune system and preserving the health of blood vessels (**Kumar *et al*., 2021).**

6. Different compounds and their uses in guava plant parts

The guava plant (*Psidium guajava*) is a traditional medicine with biologically beneficial qualities in all parts of the plant (Table 2). In the traditional medical system, it is very significant. It is a key herbal remedy for diarrhea and dysentery in Ayurveda. It is used to treat a wide range of illnesses in the system of traditional Chinese medicine. It has long been utilized to enhance human well-being **(Naga *et al*.,2022).**

The following table shows each part, its biological properties and uses.

Table 3: Different compounds and their uses in guava plant parts

Plant part	Compound	Ethno-medicinal use
Seed	Glycosides,carotenoids and phenoliccompounds	Antimicrobial activity
Bark	Phenolic	Strong antibacterial activity, stomach ache and antidiarrheal activity
Leaves	Phenolic, flavonoids, gallic acid,catechin, epicatechin,rutin, naringenin, kaempferol	Antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, anticonservative, and neuropathic activity
Skin	Phenolic	Improvement of food absorption
Pulp	Ascorbic acid, carotenoids (lycopene, β-carotene, β-cryptoxanthin)	Antioxidant, antihyperglycaemic.

7. Pharmacological properties of *Psidium guajava*

❖ Antioxidant properties

Lipid autoxidation is initiated by a sequence of lipophilic radicals. Within the biological context, hydrogen peroxide is enzymatically generated by various oxidase enzymes. Hydrogen peroxide, acting via the hydroxyl free radical, assumes the role of a signaling molecule in the synthesis and activation of inflammatory mediators.

These signaling molecules have a substantial impact on tissue damage and the development of various diseases, including diabetes, Guava boasts a substantial matter of essential antioxidants and exhibits radio-protective properties Additionally, it is enriched with a wide array of vitamins and minerals. Notably, guava contains phenolic compounds like flavonoids, as well as vital antioxidants like lycopene and flavonoids. These components contribute to the elimination of cancerous cells and the prevention of premature skin aging. Within guava leaves, quercetin is recognized as the most potent antioxidant and is dependable for its spasmolytic activity. Guava extracts, when subjected to aqueous or organic solvents, display a substantial antioxidant reservoir capable of quenching oxidation reactions. The concentration of these compounds increases proportionally with higher extract concentrations, Furthermore, pink guava exhibits pronounced antioxidant activity, Guava stands out for its exceptional richness in antioxidants, which play a pivotal role in reducing the incidence of degenerative ailments such as cognitive dysfunction, cardiovascular diseases, inflammation, cancer, arteriosclerosis, and arthritis. The therapeutic potential of guava extracts, owing to their antioxidative properties, has opened new avenues for combatting diverse complications and diseases. Further investigations are warranted to elucidate the precise mechanisms underpinning guava's antioxidant and other pharmacological activities, Remarkably, the flesh of red guava contains many distinct carotenoids, with thirteen being specifically identified as guava carotenoids responsible for its antioxidant activity **(Ashwaq *et* Enas.,2024).**

❖ Antidiabetic properties

Psidium guajava has been reported to lower the blood glucose level. Guava fruit extract has been shown to significantly restore the loss of body weight and reduces the blood glucose level in the diabetic condition. In STZ induced diabetic's guava fruit extract, when administered at a dose of 125 and 250mg/kg. Fruit extract of guava protects the pancreatic tissues, including islet beta cells, against lipid per oxidation and thus reduces the loss of insulin-positive beta cells and insulin secretion. The ethanolic stem bark extract exhibited significant hypoglycaemic activity in alloxan-induced hyperglycaemic rats at an oral dose of 250mg/kg.

Aqueous leaf extract of guava at 0.01–0.625 mg/ml showed significant inhibition on low density lipid (LDL) glycation in a dose dependent manner. Various investigations indicated that leaf extract of guava and its phenolic compounds inhibit the glycation process in an albumin/glucose model system. The guava leaf extracts also showed strong inhibitory effects on the production of Amadori products and advanced glycation end products (AGEs) from albumin in the presence of glucose **(Dev *et* Ramica.,2012).**

❖ Hepatoprotective properties

Multiple scientific investigations have provided evidence that guava leaves extract exhibits antioxidant characteristics, primarily attributable to its elevated phenolic content. This attribute is instrumental in mitigating or preventing liver damage caused by free radicals, particularly in diabetic rats induced with alloxan. Consequently, Guava leaves extract offers dual benefits of antidiabetic and hepatoprotective effects. In a previous study, it was observed that the administration of *P. guajava* fruit purée at doses of 200,400 mg/kg to rats led to a weighty drop in serum levels of AST, ALT, and alkaline phosphatase when compared to diabetic control groups. These findings suggest that *P. guajava* fruit purée is safe even at higher doses, effectively conquers hyperglycemia, and provides protection to the liver by reducing lipid peroxidation while enhancing antioxidant status in alloxan-induced diabetic rats. These results offer scientific support for considering *P. guajava* fruit purée as a nutraceutical agent for managing diabetes and hepatotoxicity. Additionally, *P. guajava* root extracts have exhibited potent hepatoprotective properties against damages induced by castor oil in rats, suggesting their potential utility in ethnomedicine, another study has highlighted the hepatoprotective actions of *P. guajava* aqueous leaf extracts and ethanolic extract, administered postoperatively at doses of 250, 500 mg/kg, underscoring their effectiveness in safeguarding liver health **(Ashwaq *et* Enas.,2024).**

- **Antibacterial properties**

The antibacterial activity of GLE has been widely studied, focusing on its effects against Gram-negative pathogens such as Escherichia coli, Klebsiella pneumoniae *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Pseudomonas aeruginosa*, *and Chromobacterium violaceum* and Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus gordonii*, *Streptococcus pyogenes*, *Bacillus cereus*, *and Bacillus anthraci* Key mechanisms include inhibiting biofilm formation, reducing bacterial adhesion, directly affecting bacterial cell membranes, inhibiting acid production, and reducing pH. Regardless of the specific effect on bacterial cell membranes caused by the extract of GLE, Dzotam and Kuete proved that the methanol extract of GLE has antibacterial properties with mechanisms that damage bacterial cell membranes, leading to the efflux of ions, proteins, and other intracellular components, thereby degrading membrane integrity and ultimately leading to bacterial cell death. These authors revealed that the extract possessed exceptional antibacterial properties against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. For Gram-positive *Staphylococcus aureus*, the most potent activity was observed (MIC: 62.5 µg/mL). On the contrary, MICs were higher for Gram-negative bacteria such as *Escherichia coli* (MIC: 125 µg/mL) and *Klebsiella pneumoniae* (MIC: 250 µg/mL). In addition, another study showed that the extract also disrupts biofilm formation through decreasing bacterial adhesion on high-density matrices, preventing extracellular polymeric substance synthesis, and lowering acid generation. The extract inhibited acid production by *Streptococcus mutans*, as these bacteria ferment carbohydrates to produce acid, hence decreasing pH and damaging enamel. The MIC for *S. mutans* was documented as 1 mg/mL. The results highlighted the strong antibacterial and antibiofilm efficacy of GLE, especially against Gram-positive bacteria and biofilm-related diseases.

The ethanol extracts of GLE prevented biofilm formation by *S. mutans* through two key mechanisms, i.e., decreasing bacterial surface hydrophobicity, which reduces adhesion, and inhibiting extracellular polysaccharide (EPS) synthesis, as reported by Phaiboon et al. At a concentration of 1 mg/mL, the ethanol extract prevented 99.34% of biofilm formation after 24 h ($p < 0.05$), but at lower doses (0.25–0.75 mg/mL), inhibition rates varied from 85% to 95%. Under sucrose-dependent conditions, bacterial attachment decreased to 1.50%, whereas under sucrose-independent conditions, it decreased to 8.43%, both at the same extract concentration of 1 mg/mL. Similarly, the mechanism of biofilm inhibition by *Psidium guajava* extract was found by Gómez et al. for periodontal pathogens, such as *Streptococcus gordonii*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. At a concentration of 1.56 mg/mL, the extract prevented biofilm formation by 77% relative to the control group ($p < 0.05$), demonstrating an effectiveness equivalent to 0.12% of chlorhexidine (positive control). Furthermore, the extract decreased bacterial adherence to host surfaces by 75% ($p < 0.05$). Kenmeni et al. found that methanolic extracts of GLE reduced biofilm development by *Bacillus cereus* and *Bacillus anthracis*. This impact was made easier by the inhibition of EPS production and the disruption of quorum sensing, a vital mechanism controlling biofilm development.

Psidium guajava also inhibits the quoruvirulent (QS) system, a key bacterial communication mechanism that regulates virulence factors. Instead of killing bacteria, QS inhibition reduces their pathogenicity by interacting with pigment synthesis. The extract significantly decreased QS-regulated pigments: pyocyanin in *Pseudomonas aeruginosa* by roughly 50% at the lowest concentration when compared to the water extract, prodigiosin in *Serratia marcescens* by 60–70% at the highest concentration, violacein in *Chromobacterium violaceum* by approximately 50% at medium and high concentrations, and staphyloxanthin in *Staphylococcus aureus* by 10–15%. Catechin (50 µg/mL), a recognized QS inhibitor, completely inhibited pigment synthesis in all examined bacteria except for *Streptococcus* pyogenes **(Hoang *et al*.,2025).**

❖ Antiviral properties

GLE contains bioactive compounds such as flavonoids, quercetin, and antiviral agents, which are therefore proposed to inhibit SARS-CoV-2 replication by targeting RNAdependent RNA polymerase (RdRp). Molecular docking analysis further revealed that phytochemicals derived from *Psidium guajava* leaves such as Longifollen and quercetin exhibited strong binding affinities with the nsP2 cysteine protease of the chikungunya virus, with minimum binding energies of -8.26 kcal/mol and -6.66 kcal/mol, respectively. GLE increased Vero cell viability by 60% compared to the virus control group, while synthesized silver nanoparticles derived from the extract increased cell viability by 40%.

Clinical studies showed significantly higher recovery rates in the GLE group compared to the control group: 49% vs. 27% at 2 weeks and 100% vs. 82% at 4 weeks, with *p*-values of 0.03 and 0.003, respectively. Consequently, the extract suggests its promise as a therapeutic agent for expediting recovery in viral infections. Furthermore, the extract has anti-HIV-1 action, targeting many phases of the virus life cycle. It blocks viral entrance by a mechanism similar to dextran sulfate and reduces HIV-1 protease activity, achieving 74.29% inhibition at a concentration of 0.085 mg/mL. In cell-associated tests, it showed an effective concentration (EC50) of 0.085 mg/mL and a selectivity index (SI) of 21.65, but in cell-free assays, the EC50 was 0.054 mg/mL with an SI of 34.07, demonstrating great effectiveness and low toxicity. These results emphasize the antiviral properties of GLE, indicating its potential as a treatment agent for anti-chikungunya and viral diseases **(Hoang *et al*.,2025).**

- **Anti-inflammatory properties**

Germ infections and production of thymus can be stopped by using guava extract in ethyl acetate It is an anti-viral mediator. It also improves mRNA expression. Guava modify the work of heme oxygenase-1 proteins. Because of this reason, it is used as an anti-inflammatory mediator. Extract of guava have anti-nociceptive properties. It is occurred by the production of acetic acid. Another key compound phenol is present in guava and it is causative mediator for the anti-allergic and anti-inflammatory activities. Guava extract doses are effective in liver damage infection as well as production of serum Ethyl acetate extract of guava leaves have the potential to decrease antigen levels. It may stop the proclamation of histamine-containing β-hexosaminidase in the RBL-2H3 cells. Therefore, the presence of IL-4 mRNA and TNF-alpha ceases. Antigen inhibits and IIB-a are spoiled in this manner. Important compounds present in guava are benzophenone and flavonoids. These amalgams are liable for the inhibition of histamine as well as for the production of nitric acid **(Faryal *et al*., 2022).**

- **Antigenotoxic and Antimutagenic properties**

Generation of DNA damage is considered to be an important initial event in carcinogenesis. A considerable number of assays exist for the detection of different genotoxic effects of compounds in experimental systems, or for investigations of exposure to genotoxic agents in environmental or occupational settings.

Treatment with an aqueous whole plant extract of *Psidium guajava*. afforded protection.

Detailed studies have revealed that pre-treatment with an aqueous guava leaf extract was found to be effective in inactivating the mutagenecity of direct acting mutagens 4-nitro-o-phenylenediamine and 2-aminofluorine in the tester strains of Salmonella typhimurium. Therefore, results showed promising antigenotoxic/antimutagenic activity. Gallocatechin isolated from the methanol extract of guava leaf also showed antimutagenic activity against E. coli **(Girish *et al.*, 2011).**

- **Anti-allergic properties**

Th1 polarization is one of the mechanisms underlying the therapeutic effects of herbalmedicine. The action of anti-allergic agents from *Psidium guajava* on T cell immunity in mice was investigated. Studies were carried out on methanol and aqueous extracts of *Psidium guajava* leaves. These extracts cause potent inhibition of histamine release from mast cells, and blocked IL- 10-mediated, *in vitro* induction of T regulatory (Tr) cells from CD4+ splenocytes of C57BL/6 mice. The extracts also shifted the Th1/Th2 balance to a Th1 dominant status by directly attenuating Tr cell activity. *Psidum guajava* leaf extracts showed anti-allergic activity on T cell immunity in mice **(Rosa *et al*., 2008)**.

8.Toxicology

This toxicologic study was conducted using dry leaves of *Psidium guajava* L. In this plant material, acute toxicologic study by the following methods: mean lethal dose LD50 test in Swiss mice and alternative toxicology (acute toxic classes) in Wistar rats. We also made the genotoxic of 2 extracts, one of aqueous type, and the other of henaxic type in an *in vitro* system of short-term somatic segregation induction assay in the *Aspergillus nidulans* fungus and an *in vivo* assay of the dry drug in mouse bone marrow micronuclei induction test. No deaths were observed in the toxicological results of the two experimental models in the dose range using up to 2 g/kg/b.w. Acute toxicity tests in rats and mice have proven the LD50 of guava leaf extracts to be more than 5 g/kg. *In vitro* genotoxicity and mutagenicity tests on *Psidium guajava* in human peripheral blood lymphocytes found no disturbances in cell division. The histological results did not suggest any damage attributable to toxicity of the studied plant material. In the *in vitro* study with *Aspergillus nidulans* D-30, results indicated a lack of genotoxic effect of these extracts, as well as in the mouse bone marrow micronucelus induction **(Rosa *et al*., 2008).**

Chapter 02: Secondary metabolites

1.Secondary metabolites:

Secondary metabolites are low molecular weight natural compounds predominantly synthesized by living organisms such as plants, fungi, and bacteria **(Bills & Gloer.,2016; Thirumurugan *et al*.,2018; Zandavar & Babazad.,2023)**.Unlike primary metabolites, which include lipids, amino acids, carbohydrates, and nucleic acids, secondary metabolites are not directly essential for the organism's fundamental processes of growth, development, or reproduction **(Nwokeji *et al*., 2016; Zandavar & Babazad., 2023)**. As early as 1891, A. Kossel distinguished them by noting that primary metabolites are ubiquitous in dividing cells, whereas secondary metabolites appear only incidentally and are not of paramount importance for the organism's life **(Thirumurugan *et al*.,2018)**. Despite this, these compounds exhibit an immense diversity in their chemical structures and biological functions **(Bills & Gloer.,2016; Zandavar & Babazad., 2023)**. Their biosynthetic pathways originate from primary metabolic routes, including the tricarboxylic acid (TCA) cycle, the methylerythritol phosphate (MEP) pathway, and the mevalonic and shikimic acid pathways **(Nwokeji *et al*., 2016)**. Secondary metabolites play crucial ecological roles in the survival of organisms, such as defense mechanisms against predators or pathogenic microorganisms **(Bills & Gloer., 2016)**. Due to their varied biological properties, secondary metabolites represent a rich source of pharmaceutically important compounds, with many having been developed into life-saving drugs and agrochemicals **(Bills & Gloer., 2016)**. They are broadly classified into major groups, including terpenes, phenolic compounds, and nitrogen-containing compounds such as alkaloids **(Nwokeji *et al*., 2016; Zandavar & Babazad., 2023)**. These valuable compounds can be extracted from plant material using various organic solvents and separation techniques like chromatography **(Nwokeji *et al*., 2016; Dhanarasu., 2012)**.

2.Phenolic Compounds

Phenolic compounds are classified as a broad and diverse group of secondary metabolites, which are organic compounds characterized by the presence of one or more benzene rings directly linked to a hydroxyl group (–OH) **(Zhang *et al*., 2022)**. This fundamental structure forms the basis for their classification into numerous subgroups based on the number of carbon rings and structural complexities. These include, but are not limited to: phenolic acids (e.g., gallic acid and caffeic acid), flavonoids (e.g., quercetin and anthocyanins), coumarins, stilbenes, lignans, and tannins (**Zhang *et al*.,**

2022; Elshafie *et al*., 2023). This extensive structural diversity highlights the vastness and widespread presence of this chemical class in the environment, particularly within the plant kingdom.

Plants are the primary source of these abundant compounds, where they are mainly synthesized via two major metabolic pathways: the shikimate pathway and the phenylpropanoid pathway **(Elshafie *et al*., 2023)**. These compounds play crucial physiological and ecological roles in plants; while not essential for basic growth and development (unlike primary metabolites such as amino acids and sugars), they effectively contribute to defense mechanisms against both biotic and abiotic stresses. For instance, they provide protection against pathogenic microorganisms, inhibit weed growth, act as attractants for pollinators, and impart distinctive colors to plants that aid in survival **(Pagare *et al*., 2015)**.

In addition to their abundance in the plant kingdom, phenolic compounds are also produced as natural products by certain microbial strains, thus expanding the scope of their biological sources **(Bruce., 2022)**. The growing interest in phenolic compounds stems from their numerous biological properties, which include potent antioxidant, anti-inflammatory, and antimicrobial activities, in addition to their anticancer and neuroimmunological properties **(Zhang *et al*., 2022)**. These health and functional benefits are attributed to their ability to neutralize free radicals and modulate cellular signaling pathways, making them compounds of potential nutritional and pharmaceutical value, and highlighting their importance in the development of new natural products for health and agriculture **(Elshafie *et al*., 2023)**.

2.1.Classification of phenolic compounds

According to carborne, these plant phenolic compounds are mainly classified (Table 4) into the following groups **(Hasanuzzaman & Nahar.,2022).**

Table4: Classification of phenolic compounds based on the number of carbons. **(Sambangi.,2022).**

Number of C atoms	Basic skeleton	Class
6	C6	Simple phenols, benzoquinones

7	C6–C1	Phenolic acids
8	C6–C2	Acetophenone,phenylacetic acid
9	C6–C3	Hydroxycinnamic acid, polypropene, coumarin, isocoumarin
10	C6–C4	Naphthoquinone
13	C6–C1–C6	Xanthone
14	C6–C2–C6	Stilbene, anthrachinone
15	C6–C3–C6	Flavonoids, isoflavonoids
18	(C6–C3) 2	Lignans, neolignans
30	(C6–C3–C6) 2	Biflavonoids
n	(C6–C3)n	Lignins
	(C6)n	Catecholmelanine
	(C6–C3–C6) n	Condensed tannins

2.2. Phenolic Acids

Phenolic acids are among the most widespread and diverse classes within plant phenolic compounds, constituting an essential part of the secondary metabolites produced by plants **(Ghasemzadeh & Ghasemzadeh., 2011; Bolat *et al.*, 2024)**. These compounds are characterized by their chemical structure, which confers multiple biological properties, rendering them of paramount importance for both plants and human health **(Ghasemzadeh & Ghasemzadeh.,2011)**. On the plant side, phenolic acids play vital roles as signaling molecules in plant-microbe symbioses, such as arbuscular mycorrhiza formation, thereby contributing to enhanced plant nutrient uptake **(Mandal *et al.*, 2010)**. As for human health, phenolic acids, abundantly present in fruits, vegetables, herbs, and spices, with daily intakes potentially exceeding 100 mg **(Russell & Duthie., 2011)**, are recognized as potent antioxidants. They act as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation **(Ghasemzadeh & Ghasemzadeh., 2011)**. Furthermore, these compounds exhibit broad biological activity, including potential anti-cancer effects **(Ghasemzadeh & Ghasemzadeh.,2011)**, and play a significant role in gut health. This is because gut bacteria can transform more complex plant phenolics into simpler phenolic metabolites, making the colon a rich source of potentially active phenolic acids that positively impact both local and systemic gut health **(Russell & Duthie., 2011)**.

2.3. Flavonoids

2.3.1. The structure and classification of flavonoids

Flavonoids are a broad and significant class of plant-derived secondary metabolites, categorized within the larger group of phenolic compounds **(Roy *et al*., 2022; Zhao *et al*., 2024)**. Flavonoids are characterized by a basic chemical structure consisting of 15 carbon atoms arranged in a C6-C3-C6 system, meaning two phenyl rings (A and B) are connected via a three-carbon bridge (C3), which is often an oxygen-containing heterocyclic ring (C ring) **(Samanta *et al*., 2011; Roy *et al*., 2022).** This structural arrangement is known as phenylbenzopyran, and it accounts for the extensive structural diversity that characterizes this class of compounds **(Yao *et al*., 2004)**. For a visual illustration of the basic structure and its main classifications, Figure 4.

Flavonoids are classified into six main types based on the oxidation level of the C ring and the C3 carbon chain: flavones, flavonols, flavanones, flavanols (catechins), anthocyanidins, and chalcones **(Yao *et al*., 2004; Roy *et al*., 2022)**. Examples of some common compounds within these types are available in (Table 5**)**.

Flavonoids are primarily synthesized in plants via the phenylpropanoid pathway and the polyketide acetate pathway, and they are universally distributed throughout the green plant kingdom, predominantly found in cellular vacuoles **(Samanta *et al*., 2011; Jimenez-Garcia *et al*., 2013).** In nature, flavonoids exist either in their free form (aglycones) or, more commonly, as glycosides, where they are linked to various sugars via glycosidic bonds, a process known as glycosylation that further enhances their structural and functional diversity **(Zhao *et al*., 2024)**.

Flavonoids play vital physiological and ecological roles in plants; they are responsible for a wide range of biological activities such as attracting pollinators by imparting colors to flowers and fruits, and aiding in seed germination and seedling development

(Samanta *et al*., 2011). They also provide protection to plants against biotic stresses (e.g., pathogens and pests) and abiotic stresses (e.g., UV radiation, drought, and heat), and act as antimicrobial agents and allelochemicals **(Samanta *et al*., 2011; Jimenez-Garcia *et al*., 2013)**. In addition to their importance to plants, flavonoids have garnered significant attention in food science and pharmacology due to their health benefits for humans, which are attributed to their potent antioxidant, anti-inflammatory, anticancer, antimicrobial, and other therapeutic activities **(Yao *et al*., 2004; Roy *et al*., 2022)**.

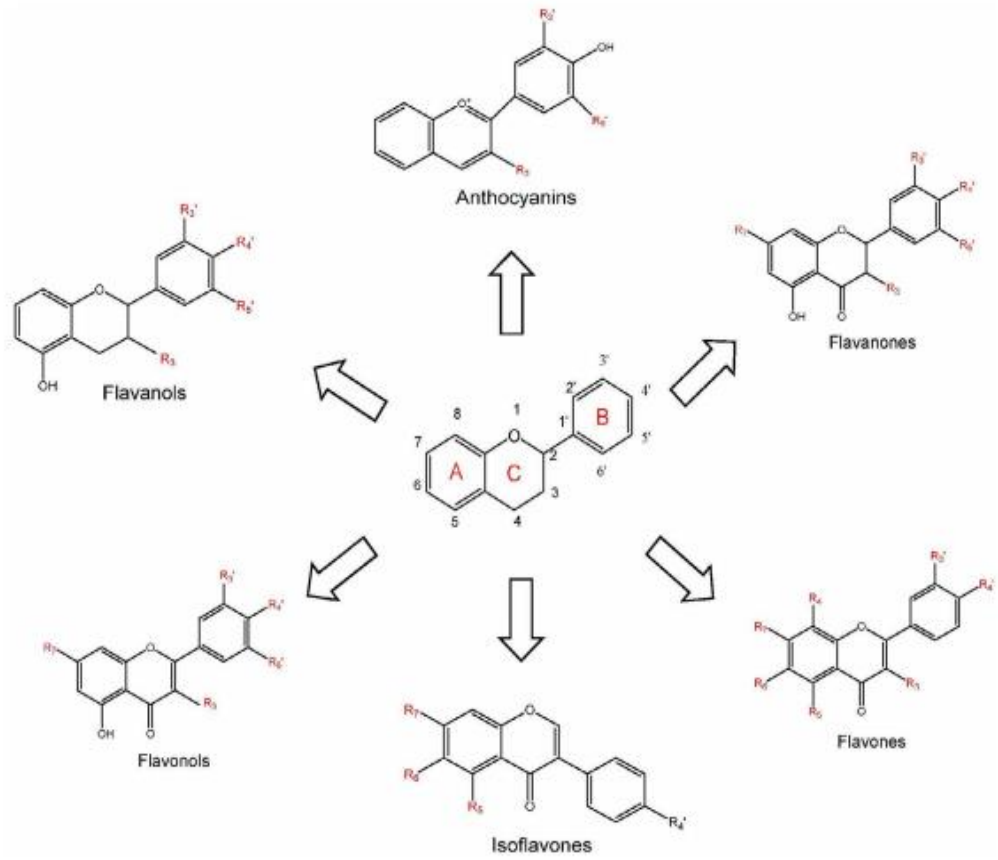

Figure 4: The structure and classification of flavonoids **(Zhao *et al*.,2024)**.

Table 5: Six types of flvonoids and some of their compounds **(Zhao *et al*.,2024)**.

Species	Name	Structure	Molecular mass
Flavones	apigenin	C15H10O5	270.27
	acacetin	C16H12O5	284.26
	baicalin	C21H18O11	446.36
	7,8-dihydroxyflvone	C15H10O4	
	luteolin	C15H10O6	

			254.24 286.27
Flavonols	quercetin	C21H20O11	448.38
	kaempferol	C15H10O6	286.27
	myricetin-3-O-B-D-glucoside	C21H20O13	480.38
	quercetin-3-galactoside	C21H20O12	464.38
	kaempferol-3-glucose-rhamnoside	C27H30O15	594.27
	quercetin 3-O-2-(6"-p-trans-coumarin)-glucose rhamnoside	C33H40O20	756.27
	quercetin-3-glucose-rhamnose-glucoside	C27H30O16	610.52
	kaempferol-3-galactoside-rhamnoside	C27H30O15	594.52
Isoflavones	Mullein	C16H12O5	284.26
	Formononetin	C16H12O4	268.30
	5,6,7,4'-tetrahydroxyflvone	C15H10O6	285.04
	5-methyl-7-hydroxyisoflvone	C16H12O3	252.27
	soy isoflvones	C15H10O2	222.27
	genistein	C15H10O5	270.27
Anthocyanins	Cyaniding	C15H11O6	287.27
	Delphinidin	C15H11O7	338.70
	morning glory pigment	C16H13O7 C16H13O6	352.72
	paeoniflrin	C17H15O7	336.72
	mallow pigment	C27H31O17	366.75

	delphinidin-3-O-arabinose		627.53
Flavanones	naringenin	$C_{15}H_{12}O_5$	272.27
	eriocidin hesperidin	$C_{27}H_{32}O_{15}$	596.54
	geraniol	$C_{28}H_{34}O_{15}$	610.56
		$C_{16}H_{12}O_6$	300.27
Flavanols	catechin	$C_{15}H_{14}O_6$	290.27
	epigallocatechin epicatechin	$C_{15}H_{14}O_7$	306.27
	epigallic acid catechin gallate	$C_{15}H_{14}O_6$	290.27
	epicatechin gallate epigallocatechin	$C_{22}H_{18}O_{11}$	458.38
		$C_{22}H_{18}O_{10}$	442.38
		$C_{22}H_{18}O_{11}$	458.37

3.Essential Oils:

According to the “*Association Française de Normalisation*” (AFNOR)and to the European Pharmacopoeia (Ph. Eur.), an essential oil isclearly defined as a manufactured product from pure, identified rawmaterials of plant origin, obtained by hydrodistillation and steamdistillation, mechanical processes (e.g., EO from *Citrus*), or by “dry”distillationfor some woods.The essential oil is then separated from the aqueous phase byphysical processes**(Tien Do *et al*.,2014).**

3.1. Chemical composition of Essential Oils (EOs):

Essential Oils (EOs) are recognized as complex and volatile mixtures of organic compounds extracted from a single botanical source, to which the distinctive aromas and flavors of plants are attributed **(Moghaddam & Mehdizadeh., 2017)**. These oils are considered plant secondary metabolites **(Eslahi *et al*., 2017)**, and their chemical composition forms the basis of their sensory and biological properties **(Başer & Buchbauer., 2010).** Figure 5 illustrates the chemical structures of some common EOs compounds**.** Terpenes dominate the chemical composition of essential oils, being the most common and diverse constituents. These terpenes are primarily composed of monoterpenes, which are compounds containing 10 carbon atoms, such as limonene and pinene, in addition to sesquiterpenes, which contain 15 carbon atoms **(Başer & Buchbauer.,2010; Moghaddam & Mehdizadeh.,2017).** Alongside terpenes, essential oils frequently contain phenylpropanoid compounds like eugenol and cinnamaldehyde, which contribute to the broad spectrum of the oil's aromatic and functional characteristics **(Moghaddam & Mehdizadeh.,2017)**. The composition can also include organosulfur compounds, as seen in garlic essential oil **(El Youssfi *et al*., 2024)**. The precise chemical composition of an essential oil is influenced by multiple factors, including the specific plant part used, geographical and climatic conditions, the extraction method, and the plant's maturity stage **(Moghaddam & Mehdizadeh., 2017)**.

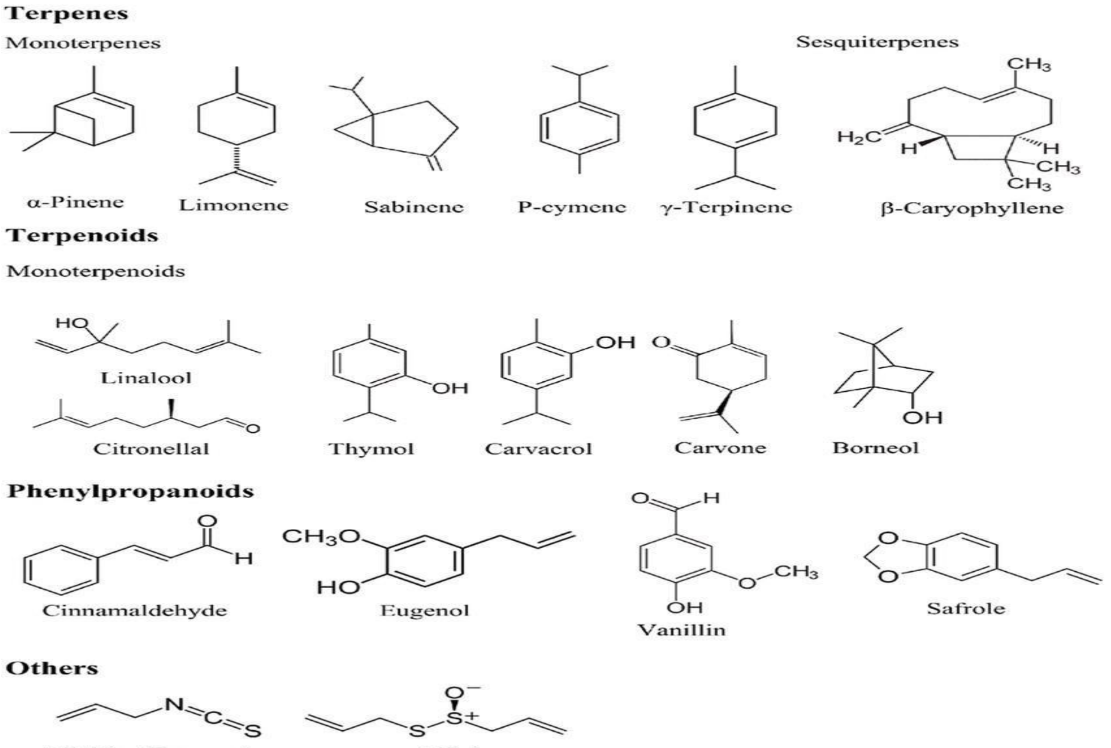

Figure 5: Chemical structures of some EOs compounds **(El Youssfi *et al*.,2024)**.

4.Alkaloids:

Alkaloids are a broad and prominent class of secondary metabolites of significant importance, characterized by the presence of at least one nitrogen atom in their structure, which is typically incorporated within a heterocyclic ring, and they exhibit alkaline (basic) properties **(Matsuura & Fett-Neto.,2015; Jain et al.,2019).** Alkaloids are defined as nitrogen-containing organic compounds primarily synthesized in plants, but they can also be found in certain microorganisms and marine animals **(Hussein &**

El-Anssary.,2018; Shoker.,2020). For a visual representation of the structural backbone of these compounds, Figure 6.

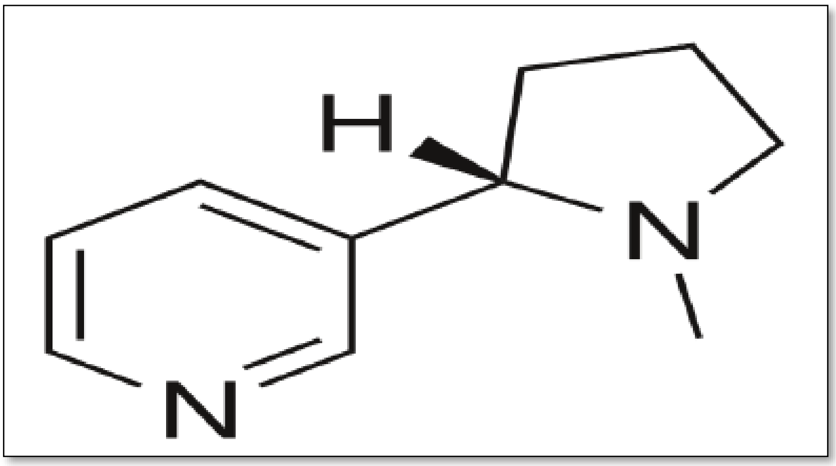

Figure 6: Alkaloids structure **(Shoker.,2020).**

Alkaloids are classified into various subgroups based on their biogenetic origin and specific biosynthetic pathway, or according to their chemical structure, such as indole alkaloids, quinolizidine alkaloids, pyridine alkaloids, isoquinoline alkaloids, and terpenoid alkaloids **(Matsuura & Fett-Neto.,2015; Jain *et al*.,2019)**. These compounds are synthesized in plants through diverse metabolic pathways and play crucial physiological and ecological roles. Although not essential for the plant's basic growth and development, they effectively contribute to defense mechanisms against herbivores and pathogenic microorganisms, owing to their toxic and deterrent properties **(Matsuura & Fett-Neto.,2015; Pagare *et al*.,2015).**

The historical and ongoing interest in alkaloids stems from their broad pharmacological and toxicological properties. Since the isolation of the first alkaloid in the 19th century, many have been utilized as effective drugs for treating a variety of diseases, such as morphine as an analgesic, quinine as an antimalarial, vincristine and vinblastine as anticancer agents, and caffeine and nicotine as neuroactive compounds **(Matsuura & Fett-Neto., 2015; Shoker., 2020)**. Their pharmacological efficacy depends on their dosage and duration of exposure, encompassing a wide range of effects on the central nervous system, which underscores their importance as biologically active compounds in the fields of medicine and pharmacology **(Matsuura & Fett-Neto., 2015).**

5.Nanotechnology

The scientific community at large has shown interest in the topic of nanotechnology. In a talk at the California Institute of Technology in December of 1959, Nobel Laureate

Richard Feynman presented the idea of nanotechnology. "Nanotechnology deals with the processing of separation, consolidation, and deformation of materials by one atom or by one molecule"**(Falke *et al*.,2024).**

5.1. Silver Nanoparticles:

Silver Nanoparticles (AgNPs) constitute a class of metallic nanomaterials, comprising silver (Ag) particles with dimensions in the nanoscale range, typically exhibiting a diameter between 1 and 100 nanometers **(Abou El-Nour *et al*.,2010; Yu *et al*.,2013)**. These particles possess fundamentally different and enhanced physical, chemical, and biological properties compared to bulk silver. These distinctions are attributed to critical factors such as an exceptionally high surface-area-to-volume ratio, their diminutive nanoscale size, and (quantum confinement effects), which influence electron behavior and confer unique optical and electrical properties **(Abou El-Nour *et al*., 2010).**

These distinctive properties, particularly their potent (antimicrobial efficacy), underscore the increasing importance of AgNPs across a broad spectrum of applications. They demonstrate activity against a diverse array of microorganisms, including bacteria, fungi, and even viruses **(Falke *et al*.,2024)**. Owing to these capabilities, AgNPs are currently utilized in numerous sectors, including disinfectants, consumer products, medical devices, and biomaterials **(Yu *et al*.,2013; Falke *et al*.,2024)**. These nanoparticles are synthesized through various methods, with (chemical reduction) emerging as an effective approach for producing stable AgNPs with precise shapes and sizes **(Desireddy *et al*.,2013)**. As their prevalence grows, studying their environmental impacts and their fate within biological systems becomes of paramount importance **(Yu *et al*., 2013).**

5.2. Advantages of Nanoparticles (Falke *et al*.,2024):

The following are some notable benefits of nanoparticles:

- Enhanced bioavailability.
- Proportionality of dose.
- Dosage forms smaller.

- Dissolves the active substance more quickly when there is a greater surface area. Increased absorption and bioavailability are typically correlated with faster dissolving rates.
- Lower dosages of drugs have less toxicity.
- Decreased variability between fed and fasting

Characterization of Nanoparticles:

- UV-visible spectroscopy.
- Fouries transform spectroscopy in the infrared.
- Analysis of X-ray diffraction.
- Electron microscope for transmission.
- Electron microscopy.
- Mass spectrometry.
- Electron microscopy using transmission.

Microscopy using scanning electron.

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