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FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE

DEPARTEMENT DES SCIENCES AGRONOMIQUES

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Intitulé :

Valorization of Plant Products

Destiné aux étudiants de 3ème Année Licence

Spécialité : Biologie et Physiologie Végétale

Réalisé Par

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MCA en Biologie & Physiologie végétale

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Table of Content

COURSE OUTLINE (Syllabus)

Title of the Degree: Plant Biology and Physiology (PBP 3rd Year)

Semester: 02

Course Title: Valorization of Plant Products

Code: VPP

Teaching unit: Methodological

Credits: 05

Coefficient: 03

Course Outcomes:

- Learn the theoretical and practical concepts related to the cultivation, extraction, and formulation of plant products.
- Understand the main natural resources and plant metabolites.
- Be aware of the various applications of plant extracts in the food, pharmaceutical, and cosmetic industries.

Course Content:

Course:

Chapter 1. Introduction

Chapter 2. Plant secondary metabolites and their main sources

Chapter 3. Valorization of natural plant substances:

3.1. Agri-food valorization: Aromatic plants, spices and condiments; phytonutrients; vegetable pigments, etc.

3.2. Pharmaceutical valorization: Medicines based on isolated natural substances, Medicines based on plant extracts, homeopathic medicines: manufacture - standardization.

3.3. Cosmetic valorization: Manufacture of soaps, creams, perfumes, etc.

Practical works/Tutorials:

1. Extraction of secondary metabolites.
2. Biological activities of secondary metabolites.

CHAPTER I
Introduction

CHAPTER I
Introduction

Learning Outcomes:
By the end of this chapter, students should be able to:
- Explain the concept of "Valorization of plant products."
- Describe the key characteristics of true plants.
- Highlight the significance of plants.
- Categorize the types of crops according to their uses.
- Ect.

1. What do we mean by the "Valorization of plant products"?

The "valorization of plant products" refers to the process of enhancing or increasing the value or utility of a wide range of bioactive compounds (BACs) (e.g. polyphenols, carotenoids, etc.), This involves converting raw plant materials or by- products into higher-value products through innovative processing methods, technological advancements, and the development of new applications for plant-based materials. The aim is to maximize the economic, environmental, and social benefits derived from these natural resources (Pavlić *et al*., 2023).

Valorization can take many forms, including:

➢ **Extraction of valuable compounds**: Plants contain a wide array of bioactive compounds with pharmaceutical, nutraceutical, and industrial applications. Valorization involves extracting these compounds, such as antioxidants, essential oils, and phytochemicals, for use in sectors such as healthcare, cosmetics, and food production (Rahman, 2021).

➢ **Development of biobased materials:** Plant biomass can be utilized to create biodegradable materials, biofuels, and bioplastics as sustainable alternatives to conventional petroleum- based products. Valorization efforts focus on optimizing processes to convert plant biomass into these materials efficiently and cost-effectively (Falua *et al*., 2022).

➢ **Utilization of agricultural residues:** Valorization involves finding innovative uses for agricultural residues, such as crop residues, husks, and pomace, which are often discarded as waste. These residues can be converted into biofuels, animal feed, fertilizers, or used in biorefinery processes to extract valuable chemicals and compounds (Ginni *et al*., 2021).

➢ **Creation of functional foods and nutraceuticals:** Valorization aims to capitalize on the nutritional and health-promoting properties of plant-based ingredients by developing functional foods, dietary supplements, and nutraceuticals. This involves formulating products enriched with vitamins, minerals, antioxidants, and other plant-bioactive compounds (Rao *et al*., 2022).

➢ etc.

Overall, valorization of plant products involves finding innovative ways to use, enhance, and sustain plant-derived materials economically, environmentally, and socially... etc.

2. What is a plant?

The definition of ‘’*what constitutes a plant*’’ has exercised the minds of people since ancient times. An obvious way of categorizing living organisms is to divide them into things that move (animals) and things that do not (plants). This was the system originally adopted by the ancient Greeks and was commonly used until modern times. However, according to this definition fungi would be included in the same group as plants simply because both were sessile (motionless) organisms. Thanks to DNA sequence analysis, we now know that fungi are phylogenetically much closer to animals than to plants. Therefore, in modern classification systems plants, animals and fungi are placed in separate groups or taxa (Murphy, 2011).

Unfortunately, the convenient division between plants, animals and fungi breaks down when we consider some of the many microorganisms that are clearly plant-like (they can photosynthesize) but also have animal characteristics (they are motile and can even ingest smaller cells as prey).

For example, some unicellular green algae, such as Euglena, can use light to photosynthesize just like higher plants. However, in reduced light these algae can become heterotrophic and use their flagella to move around and hunt for prey similarly to animals.

To complicate matters further, although the capacity for photosynthesis is generally regarded as the defining feature of a plant-like organism, there are numerous non-photosynthetic plants.

For example, several groups of angiosperms, such as some forms of dodder (e.g. Cuscuta spp.) and some orchids (e.g. Neottia nidusavis), have completely lost the ability to photosynthesize as a result of their parasitic lifestyles. These organisms are obviously plants but, because they cannot photosynthesize, they now lack one of the fundamental characteristics that would normally define them as plants.

In reality, there is no simple way to define a plant and there will always be exceptions to any definition that we can come up with. So-called 'true plants' are usually defined as members of



the phylum Plantae. These organisms are normally multicellular, sessile, eukaryotic autotrophs that derive their main nourishment from oxygenic photosynthesis, using water as an electron donor and atmospheric CO_2, as a carbon source. The vast majority of 'true plants' are terrestrial, although several have secondarily become aquatic. True plants fall into two groups:

- The ‘***’lower plants’’*** that range from relatively simple non-vascular species such as liverworts and mosses to seedless vascular plants like the ferns.
- The seed-bearing ***‘’higher plants’’*** that include the gymnosperms and angiosperms and are the most widespread, complex, diverse and successful groups of true plants.

Although higher plants (especially Angiosperms) are by far the most important group, several other types of plant-like organisms are also useful, especially in a biotechnological context. The two main groups of photosynthetic organisms that are not defined as 'true plants' are the algae and cyanobacteria. The algae, all of which are aquatic eukaryotes, range from simple phytoplankton species to more complex multicellular organisms such as the green, brown and red seaweeds often seen on beaches at low tide. The algae also include many unicellular species, known as microalgae, all of which are eukaryotes. The final group of plant-like organisms is the cyanobacteria, which are the only group of prokaryotes capable of photosynthesis (**Figure 1**).

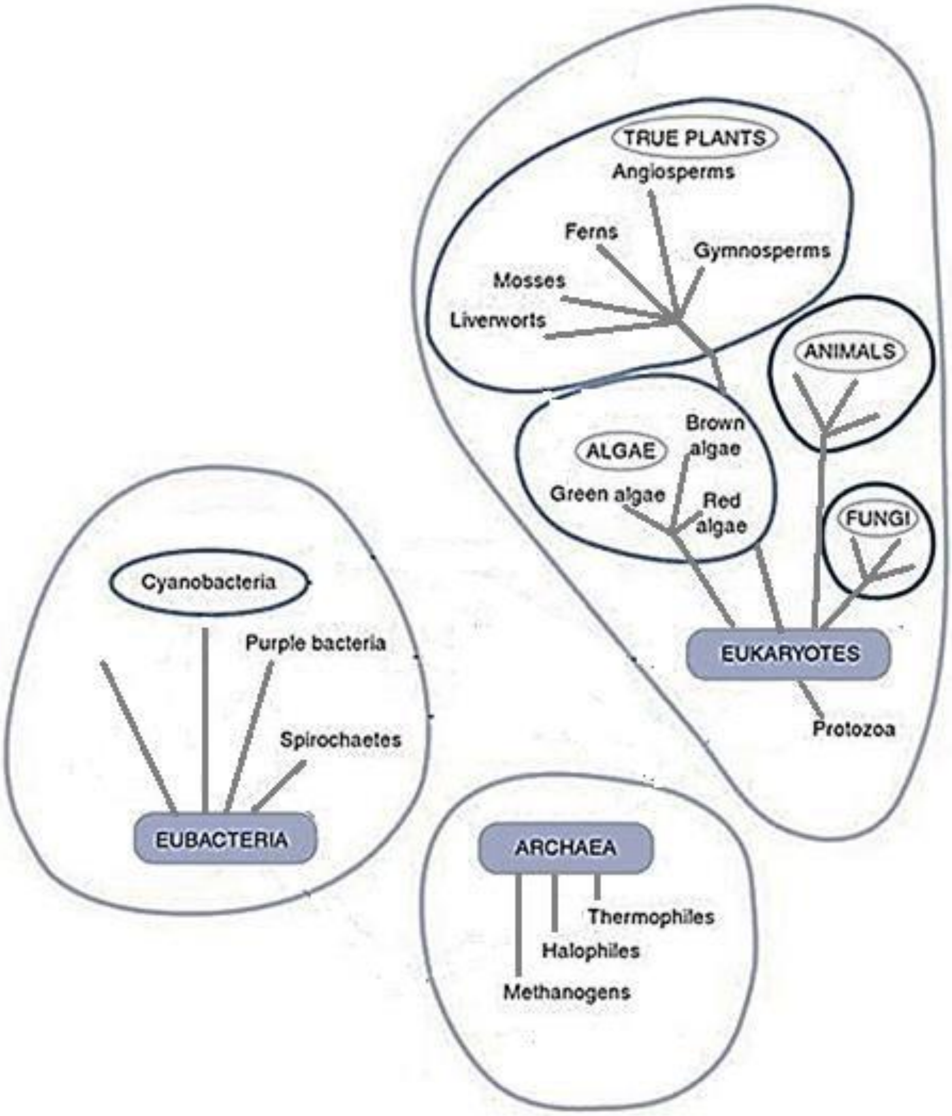


Figure 1. The major groups of plants and plant-like organisms. There are three major groups of plant-like organisms. The largest group is the so-called "true plants" that dominate most terrestrial ecosystems and provide the vast majority of crops and other useful plants that are exploited by people. The second major group is the algae, a diverse collection of photosynthetic eukaryotes, some of which have been long exploited by coastal communities. The third group is the cyanobacteria, which are the only prokaryotes capable of oxygenic photosynthesis. Several microalgae and cyanobacteria are currently being biotechnologically manipulated to act as bioreactors or sources of renewable biofuels. (Adopted from Murphy, 2011).

3. What are the main features of a 'true plant'?

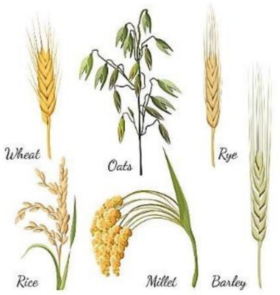
The specific features of a 'true plant' are:

- To be EUKARYOTIC (true nucleus).
- To have cells surrounded by WALLS made of CELLULOSE.
- To store their organic reserves as STARCH.
- To do PHOTOSYNTHESIS.

4. What are the different categories of crops?

Plants can be divided into wild plants and crop plants (crops). It is known that humans began using plants more than 100.000 years ago, but they only began cultivating plants (crops) 10.000 years ago when climate conditions were favorable (Murphy, 2011).

A crop is a plant or plant product that can be cultivated and harvested for profit or subsistence. By use, crops fall into six categories: food crops, feed crops, fiber crops, oil crops, ornamental crops, and industrial crops (National Geographic Society, 2023).



a. Food Crops:

Food crops were historically the first to be cultivated and harvested. They are grown for human consumption. Food crops, particularly seeds (grains), are strategically important.

The following are the types of food crops:

❖ **Seeds:**

They include several types of field crops, such as cereals, nuts, legumes, and some spices. Seeds are high in fiber, fats, vitamins, minerals, and antioxidants.

Cereals include wheat, rice, barley, millet, oats, rye, sorghum, and more. They are a rich source of starch, protein, dietary fiber, and nutrients, which makes them an integral part of the daily diet. In particular, maize, rice, and wheat provide the most calories and protein consumed in developing countries.

❖ **Fruits:**

Fruits include apples, pears, citrus, stone fruits, tropical and exotic fruits, berries, and more. They contain a lot of dietary fiber, vitamins, minerals, and antioxidants, such as flavonoids, which promote good health.

❖ **Vegetables:**

Vegetables are high in water content and low in calories. They are also rich in dietary fiber, antioxidants, minerals, and vitamins (especially A and C).

There are several types of vegetable crops:

❖ **root vegetables:** beets, carrots, sweet potatoes, turnips;

❖ **tubers:** potatoes, yams; etc.

❖ **stem vegetables:** asparagus, celery, fennel;

❖ **leafy green**: lettuce, spinach, silverbeet; etc.

❖ **allium or bulb vegetables:** garlic, leeks, onions, shallots;

❖ **head or flower vegetables:** artichokes, cabbage, cauliflower;

❖ **cucumber family vegetables:** pumpkin, cucumber, zucchini (courgette).

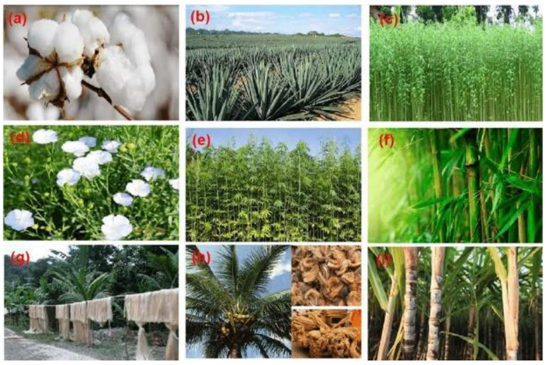
❖ **Spices:**

Spices fall into two types: the spice seeds (pepper, ginger, etc.), and herbs (coriander, parsley, etc.). Spices are used sparingly to enhance food flavor and aroma. They contain essential oils and alkaloids that aid appetite and digestion (thyme, cinnamon, oregano, rosemary, etc.) (Ahmed *et al*., 2023).

b. Forage or Feed Crops

Forage, plants contain nutrients that animals require for development. They are grown for livestock consumption and are essential in pasture management. Some of the most crucial types of forage crops are sorghum, alfalfa, barley, oats, millet, soybeans, wheat, and maize.

c. Fiber Crops



Plants grown to produce fiber for textiles, cordage, filling, and paper are known as a fiber type of crops. The well-known fiber plants are cotton, hemp, jute, flax and nettle. Some of them have a good prospect as agricultural biomass with the potential of being converted to ethanol or biofuel.

d. Oil Crops

Thanks to technological advances over the last century, plants can be processed and broken down into their primary components, including oil. Today, the oil type of crops is the second most important determinant of the agricultural economy (after cereals) and is the third largest user of farmland (Ortiz *et al*., 2020).

The essential plants of this type are soybean, sunflower, rapeseed, canola, and peanuts. They are high in oils, dietary fibers, proteins, minerals, and vitamins. Apart from producing oil for human consumption, this type of plants is used in various industries, including soaps, paints, machinery lubricants, fuel, and many more.

Oil plants also supply the raw materials for biodiesel production. Soybean, rapeseed, sunflower, camelina, and palm are typical biodiesel sources (Yan *et al*., 2023).

e. Ornamental Crops

Plants grown for decorative purposes in parks, gardens, and landscaping design projects are related to the ornamental type. Ivy, oleander, holly, tulips, and azaleas are common decorative plants (De Pascale & Romano, 2017).

f. Industrial Crops

Industrial plants are a group of crops that although grown commercially for products of economic value, have limited value for food or feed. Examples of industrial crops are cotton, flax, jute, sugarcane, sugar-beet, coffee, tea, tobacco, coconut, and soybeans. On the other hand, many feed crops such as maize are also an important feedstock for several industrial products, including bioplastic and biofuel (Singh, 2010).

5. How are plants important to humans?

Plants are God's gift as they are beneficial for us in many ways. Without plants, there would be no more life existing on planet earth.

Plants provide us with a variety of things to fulfil our daily requirements, including food to eat, air to breathe, clothes to cover our body, wood, medicine, shelter, and many products for human benefit. Plants are the primary producers, and all other living organisms on this planet depend on plants (Baluška & Mancuso, 2020).

Plants benefit us by reducing both physiological and psychological stress, such as:

- Improves mood
- Boosts air quality
- Decreases risk of illness
- Encourages healthy eating
- Relieves stress and anxiety.

Problems Chapter 1.

1. Can life exist without plants?
2. Give five reasons why plants are essential to life on earth.
3. What are the benefits of plants to humans and the environment?
4. Why do we need to improve the value of plant-derived materials and how can we do it?
5. What are the six categories of crops?

CHAPTER II

Plant Secondary metabolites and their main sources

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Plant Secondary metabolites and their main sources

Learning Outcomes:
By the end of this chapter, students should be able to:

- Differentiate between primary and secondary metabolites.
- Describe the various types of secondary metabolites.
- Explain the main functions of secondary metabolites.
- Identify the primary species that produce secondary metabolites.
- Provide at least three examples of the medicinal value of secondary metabolites.
- Define allelopathy.
- Demonstrate additional relevant knowledge.

1. Introduction

The **metabolism** can be defined as the sum of all the biochemical reactions carried out by an organism. Metabolites are the intermediates and products of metabolism (Thirumurugan *et al*., 2018). Most of the carbon, nitrogen and energy metabolites are ends up in molecules that are common to all cells and are required for the proper functioning of cells and organisms. These molecules (Carbohydrates, proteins, lipids, and nucleic acids) are called **primary metabolites (PMs).**

Unlike animals, however, most plants divert a significant proportion of assimilated carbon and energy to the synthesis of organic molecules that may have no obvious role in normal cell function. These **small molecules** are called **secondary metabolites (SMs)** by Kossel in 1891 (Thirumurugan *et al*., 2018).

While the distinction between primary and secondary metabolites is not always easily made, since they share the same intermediates and are derived from the core metabolic pathways (**Figure 2**). PSMs generally occur in relatively low quantities and their production may be widespread or restricted to particular families, genera, or even species. Also known as ***natural products***, these ***phytochemicals*** (~70% from about 250.000 SMs) were known to have significant **economic and medicinal value (**Hartmann, 2007).

Natural products have found use in antiquity as folk remedies, soaps[1], and essences. They include drugs[2] and other medicinal products, dyestuffs[3], feedstocks[4] for chemical industries (gums[5],

1 a substance used with water for washing and cleaning, made of a compound of natural oils or fats with sodium hydroxide or another strong alkali, and typically having perfume and coloring added.
2 a medicine or other substance, which has a physiological effect when ingested or otherwise, introduced into the body.
3 a substance that yields a dye or that can be used as a dye, especially when in solution.
4 raw material to supply or fuel a machine or industrial process.
5 a viscous secretion of some trees and shrubs that hardens on drying but is soluble in water, and from which adhesives and other products are made.

resins[6], rubber), and a variety of substances used to flavor food and drink. In recent years, however, it has become increasingly evident that many natural products do have significant ecological functions, such as protection against microbial or insect attacks.

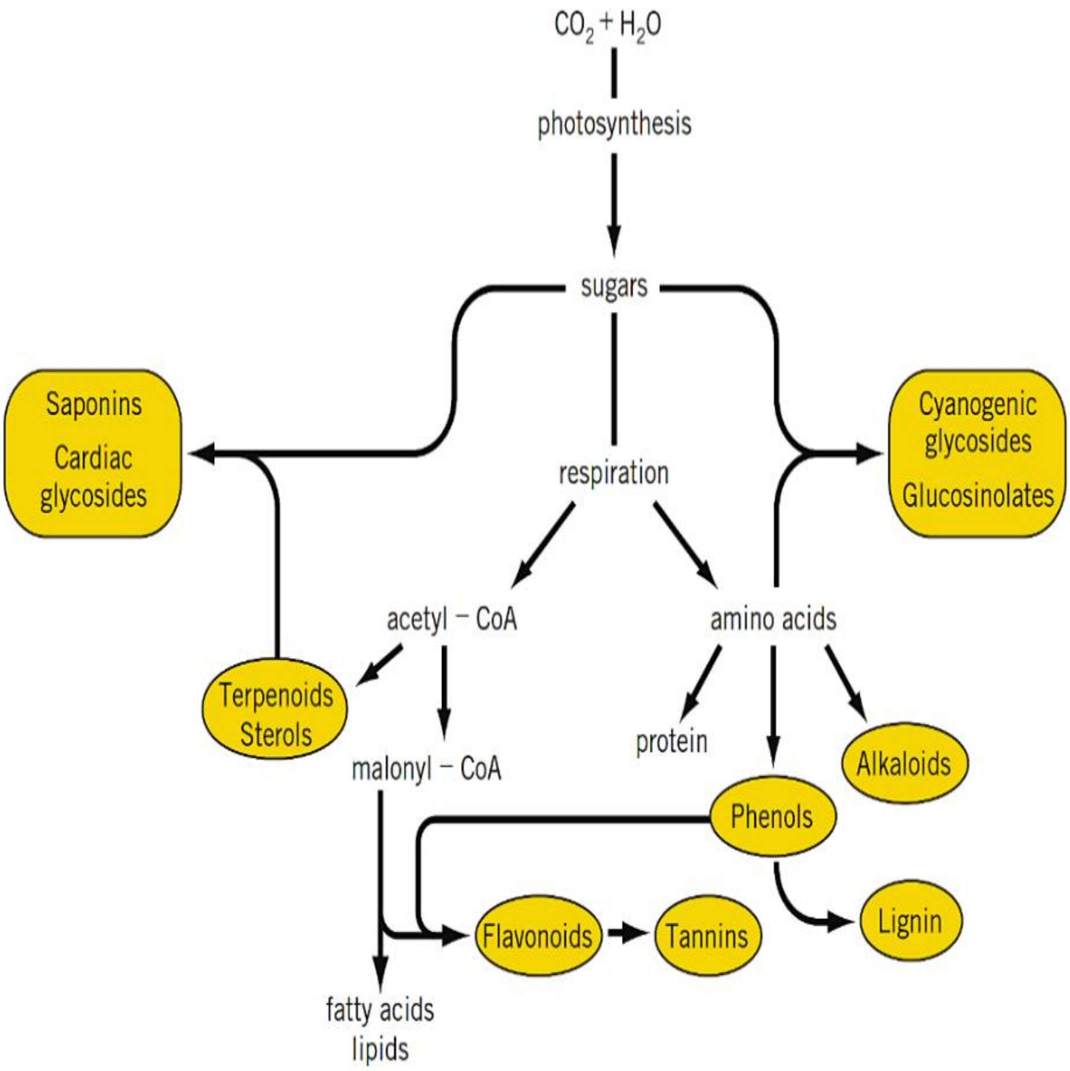

Figure 2. A diagram illustrating biosynthetic relationships between the major primary and secondary metabolites (circled) (After Singh & Patel, 2024).

2. Plants secondary metabolites

A Plant can be considered as a storehouse of a huge number of chemicals biosynthesized by many metabolic pathways like photosynthesis, glycolysis, Kreb's cycle, shikimic acid pathway, mevalonic acid pathway, etc. Primary metabolites include sugars, citric acid, Kreb's cycle intermediates, amino acids, protein, nucleic acid, and polysaccharides (Kandar, 2021).

6 a sticky flammable organic substance, insoluble in water, exuded by some trees and other plants (notably fir and pine).

About 100,000 of these low molecular weight compounds are reported from different plants so far. Many of SMs are widely distributed among angiosperm families, however, relatively complex compounds are only restricted to certain genera.

Some evidence suggests that plants produce these natural products in response to many environmental pressures (stresses), in order, at least:

- to protect themselves from herbivores and pathogens,
- to compete with other species,
- to improve their reproductive fitness.

In this section, the major sources of important PSMs and their functions will be discussed.

2.1. Terpenoids:

Terpenoid biosynthesis in plastids (**Figure 3**) results in the formation of more than **25,000** terpenoids. They are known to be synthesized within different tissues of economically important plants (Morrone *et al*., 2011). Rubber is one of the largest known terpenoids, which contains almost 400 to 100,000 isoprene units. About 1800 species of plants are known to synthesize rubber among which *Hevea brasiliensis* (*Euphorbiaceae*) is most common, which synthesizes rubber in the form of milky latex in laticiferous cells.

Aromatic monoterpenoids are mostly essential oils which are volatile organic compounds biosynthesized within plastids of parenchymatous cells of many flowers and leaves. They are secreted by glandular trichomes to attract pollinators, as many insects are color-blind and are only attracted towards the plants due to their fragrance; many flowers stop emitting fragrance once pollination is done.

Monocots, like wheat, oat and barley, release aromatic oils in response to pathogen attacks by fungi, and therefore, their concentration is high in infected tissues.

Another important role of many essential oils involves the removal of toxic waste products during translocation within the plant body, thus acting as natural cleansers.

However, some terpenoids are produced only in response to bacterial or fungal infection, and also act as allelochemicals. Many industries, like food, pharmaceutical, perfumery and cosmetics, depend on aromatic and antibacterial properties of aromatic terpenoids. They are used in beverages, vitamins (A, D and E), rubber and gutta-percha (ex., used in dental surgery). They also have a role in horticulture and floriculture where they make pigments of yellow-orange shades, that is, carotenoids. Monocots like other angiosperms have evolved many mechanisms which help to survive against many pathogen attacks, and increase their chances of survival.

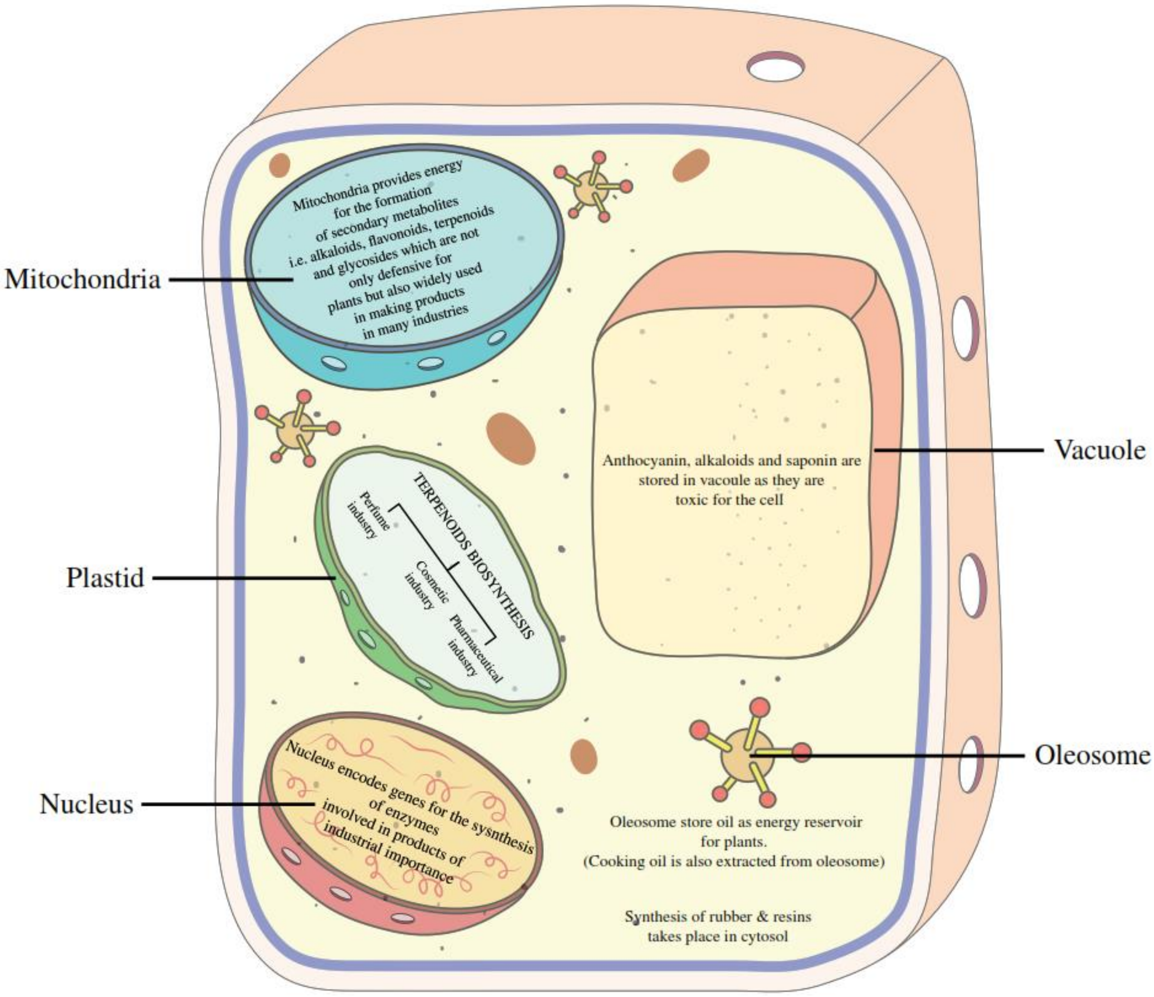

Figure 3. A plant cell from an industrial perspective showing different sites for the synthesis of products of industrial importance, which include secondary metabolites like alkaloids, terpenoids and flavonoids (After Khan, 2017).

Many members of the family *Poaceae* are known to synthesize alkaloids, flavonoids, glycosides, phenolic compounds, tannins, steroids and triterpenoids (Ankanna *et al*., 2012). In addition to this, families like ***Aliaceae***, ***Agavaceae***, ***Arecaceae***, ***Commelinaceae***, ***Liliaceae***, ***Musaceae*** and ***Zingiberaceae*** also comprise many of these metabolites. **Benzoxazinoids** are tryptophan-based defensive compounds synthesized mostly in young leaves of cereals like wheat, maize, rice, barley and oat. Due to their toxic effects, plants store them in vacuoles or in plastids in inactive glucoside form. Volatile oils from lemon grasses and vanilla species make important contribution in food and pharmaceutical industries.

2.2. Alkaloids:

A common property of alkaloids is their bitter taste, like atropine[7], piperine[8], pure caffeine, nicotine, cocaine, morphine, quinine (*Cinchona bark*[9]), scopolamine and solanine[10]. Many of them are psychoactive and act as neurotransmitters[11] (**Table 1**).

Alkaloids are regarded as reserve materials for protein synthesis, as protective substances discouraging animal or insect attacks (*xylophagous larvae*). They are also known to influence chromosome stability and inhibition of DNA duplication, which can also be toxic for other plants (see Box 1 for examples).

Families such us *Fabaceae*[12] and *Solanaceae*[13] are well known sources of alkaloids. Some of them are poisonous like deadly nightshade (*Atropa belladona*) and poison hemlock (*Conium maculatum*) from the *Apiaceae*[14] family.

Alkaloids are basically classified into **tropane alkaloids TA** (tyrosine derivatives) or **monoterpenoids indole alkaloids MIA** (tryptophan derivatives).

- More than 200 tropane alkaloids (TA) are reported in many plants and many genera from families *Brassicaceae*, *Convolvulaceae*, *Erythroxylaceae*, *Solanaceae*, *Proteaceae* and *Rhizophoraceae*.
- More than 2500 MIA are reported in angiosperms. Among them, vinblastine and vincristine, along with almost 150 MIAs are specifically expressed in *Catharanthus roseus* (*Apocynaceae*[15]), and are currently being used in cancer therapy (**Figure 3**).
  - Ajmaline is produced by *Rauvolfia serpentine* (*Apocynaceae*) and the anticancer compound camptothecin is mostly produced in *Camptotheca acuminata* (*Nyssaceae*).
  - Catharanthine is found to have anti-fungal activity on leaf surface.
  - Many other MIA-like secoiridoids are reported to be potent herbivores deterrents in families like *Apocynaceae*, *Gentianaceae* and *Rubiaceae*[16].

7 Atropine is a tropane alkaloid found in various plants of the Solanaceae family, such as belladonna, datura, henbane and mandrake.
8 Antipyretics, a drug used to prevent or reduce fever.
9 *Cinchona bark* contains quinine, which is a medicine used to treat malaria.
10 Solanine is a toxic glycoalkaloid known to accumulate under certain conditions in potato plant, sprouts and tubers in levels that if ingested, may cause poisoning in humans and farm animals.
11 Cause addiction (fr= toxicomanie, Ar= إدمان).
12 The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, are a large and agriculturally important family of flowering plants. (765 genera and 20.000 species).
13 It includes a number of agricultural crops, medicinal plants, spices, weeds, and ornamentals. (98 genera and 2.700 species).
14 Family of celery and carrot. It contains more than 3,800 species in about 446 genera.
15 Commonly known as the dog-bane family (الفصيلة الدفلية), ex. Nerium oleander.
16 Commonly known as the coffee family. It contains about 13,500 species in about 620 genera.

- Alkaloids, like caffeine and nicotine, have natural pesticidal properties, and transgenic plants are being synthesized using tobacco in order to increase plant's resistance against pathogen infections (Kim & Sano, 2008).
- *Calotropis procera* (*Apocynaceae*) is known to possess many alkaloids like calotropin, calotaxein and uskerin that have potential to stimulate cardiovascular system.

Table 1. Example of some alkaloids in plants and their clinical use.

Compounds	Source	Plant Family	Effects and uses
Morphine	*Papaver somniferum*	*Papaveraceae*	Analgesic
Codeine	*Papaver somniferum*	*Papaveraceae*	Analgesic, antitussive
Vinblastine	*Catharanthus roseus*	*Apocynaceae*	Anticancer
Camptothecin	*Camptotheca acuminata*	*Nyssaceae*	Anticancer
Atropine	*Hyoscyamus niger*	*Solanaceae*	Prevention of intestinal spasms[17], antidote
Caffeine	*Coffea arabica*	*Rubiaceae*	Stimulant, natural pesticides
Nicotine	*Nicotiana tabacum*	*Solanaceae*	Stimulant, tranquillizer
Cocaine	*Erythroxylum coca*	*Erythroxylaceae*	Local anesthetic.

Figure 3. Alkaloids give bitter taste to plant organs where they are synthesized. *Catharanthus roseus* (Madagascar periwinkle) is a dicot herb with anti-carcinogenic and anti-diabetic potential due to alkaloids like catharanthine (Khan, 2017).

2.3. Phenolic compounds:

Phenolic compounds (8000 molecules) are secondary metabolites, which are produced in the **shikimic acid** of plants and **pentose phosphate**. They contain benzene rings, with one or more **hydroxyl substituents** (**phenol units**), and range from simple phenolic molecules to highly polymerized compounds. Based on their chemical structures, phenolic compounds can be divided into different subgroups, such as phenolic acids, flavonoids, tannins, etc. (**Table 2, Figure 4**).

As with other secondary products, many phenolic compounds appear to be involved in plant/herbivore interactions. Some (e.g., lignin) are important structural components, while others appear to be simply metabolic end-products with no obvious function.

17 a sudden involuntary muscular contraction or convulsive movement.

As natural antioxidants, phenolic compounds are found abundantly in plant food and beverages, which play vital parts in healthcare. It is well-known that normally consumed fresh and processed fruits, for instance raspberries, apples, grapes, pears, strawberries and their derived products, like juices and jams, are the major sources of phenolic compounds (Pento *et al*., 2007).

Table 2. Bioactive properties of the phenolic compounds

Phenolic compound	Main sources	Bioactivities
PHENOLIC ACIDS		
Hydroxybenzoic acids		
Gallic acid	Grapes, tea, walnut, cashew nut, hazelnut …	Antioxidant, antimicrobial, anti-inflammatory, antitumoral, and neuroprotective activities.
Ellagic acid,	Berries, rye, mustard and vegetables	
Hydroxycinnamic acids		
Ferulic acid	Cereal, fruits, mushrooms	
Caffeic acid	Coffee, mushrooms	
p-Coumaric acid	Carrot, garlic, grapes, tomatoes	
FLAVONOIDS		
Flavonols		
Kaempferol	Cabbage, cauliflower, spinach	Antioxidant, anti-inflammatory, antitumoral, and cardioprotective activities.
Quercetin and derivatives	Apples, berries, onion and pears	
Myricetin and derivatives	Berries, herbs and vegetables	Antioxidant, anti-inflammatory, and antitumoral activities.
Flavones		
Apigenin	Fruits and vegetables	Anti-inflammatory, antiproliferative, and antitumoral activities.
Tangeretin	Citrus fruits	
Luteolin	Broccoli, celery and green peppers	
Flavanols		
Catechin and its derivatives	Berries, cacao and green tea	Antioxidant and antimicrobial activities, Anti-inflammatory, anti-obesity, cardioprotective activities.
Flavanones		
Hesperidin	Citrus fruits	Antioxidant, anti-inflammatory, antitumoral, antiallergic activities.
Naringin	Citrus fruits and grapefruit	Antioxidant, anti-inflammatory, anticancer, anti-lipidemic, antibacterial activities.
Taxifolin	Bark (*Pinus* or *Larix*) and seeds (*Silybum*)	Antioxidant, anti-inflammatory, antiviral, antibacterial, anticancer, neuroprotective activities.
Isoflavones	Soybean and soy products	Cardioprotective activity, estrogenic effects.
Anthocyanins	Berries, grapes and purple carrot	Antimicrobial, anti-inflammatory, antioxidant, anti-proliferative, cardioprotective activities.
TANNINS		
Epigallocatechin gallate	Apples, berries and cacao	Antioxidant, antimicrobial, and antitumoral activities.
Gallotannin	Grapes, apples, pears, plums, peaches	
STILBENES		
Resveratrol	Berries, grapes and peanuts	Antioxidant, anti-inflammatory, and cardioprotective activities.
LIGNANS	Flaxseed and sesame	Antioxidant, anticancer, anti-inflammatory, neuroprotective, and antimicrobial activities.

(Adapted from Albuquerque *et al*., 2021)

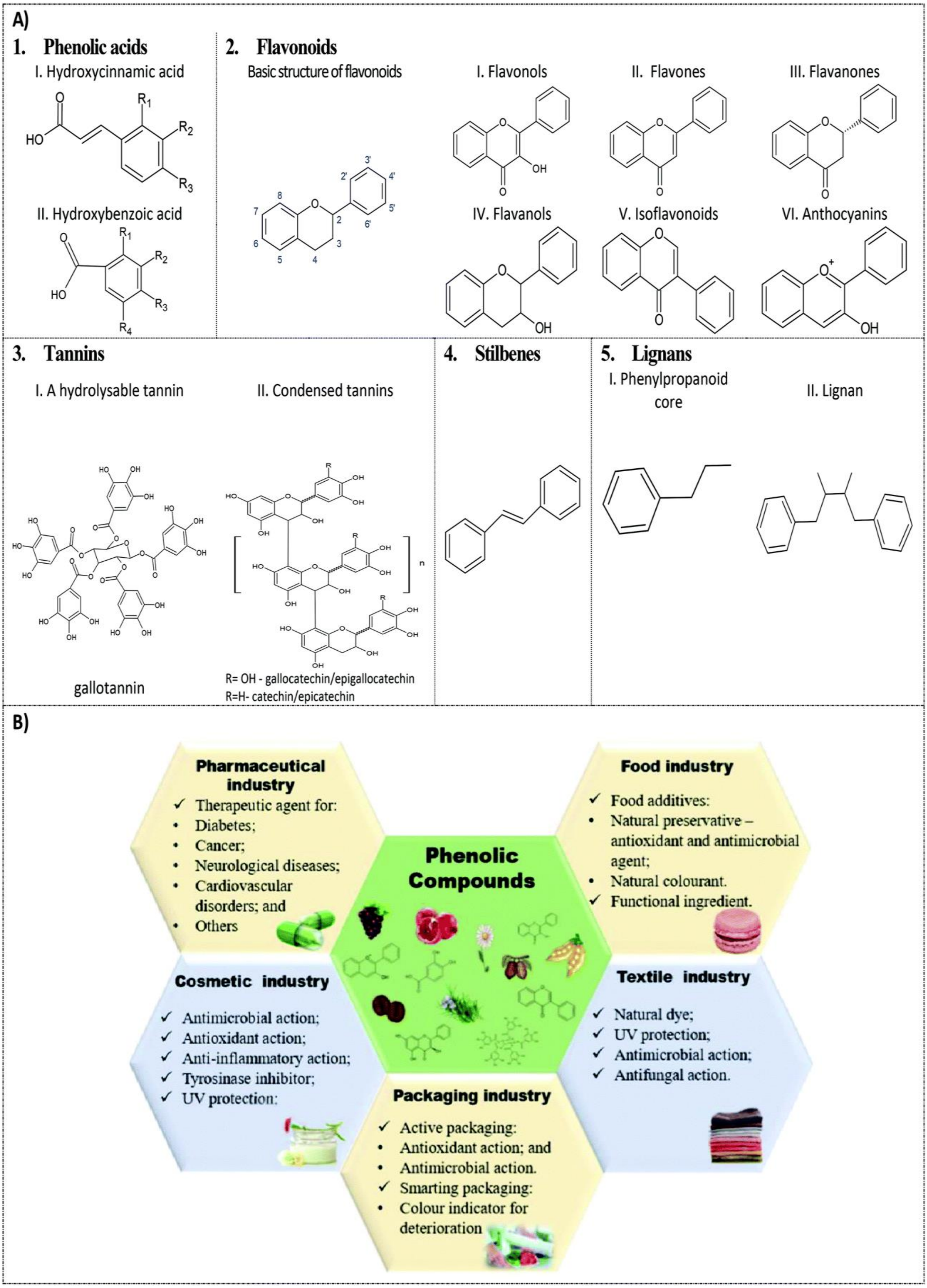

Figure 4. Main phenolic compounds and their industrial application. (A) Chemical structures. (B) Industrial applications (After Albuquerque et al., 2021).

- **Flavonoids** are important phenolic compounds, which form plant pigments, required for:
  - *pollination and pollen fertility,*
  - *auxin transport,*
  - *defense against UV-B radiations,*
  - *prevention of photooxidative damage,*
  - *protection against pathogen infection, stress conditions*
  - *protection against oxidative stress*
  - *signaling during nodulation.*
  - allelochemical reactions against many herbivores, pathogens and other plants.
  - nutrient retrieval during senescence.
- More than 6000 flavonoids are identified in plants, and that number is expected to increase in the future (Ferrer *et al*., 2008). They give different colors to flowers, fruits and seeds.
- They are classified in divers' groups such as: **chalcones, flavones, flavonols, flavandiols, anthocyanins, proanthocyanidins (condensed tannins).** Legumes (*Fabaceae*) and some non-legume plants synthesize a specialized flavonoid, that is, isoflavonoid (Wang, 2011). Grapes and peanuts synthesize **stilbene**, which is closely related to chalcone. Flavonols are important flavonoids in stress response. Isoflavones are insecticidal and anti-fungal compounds.
- Many flavonoids form **white** and **light-yellow** color in flowers and leaves. Leaves contain flavones and xanthophylls but their effect is masked by **green chlorophyll**. They absorb light in UV region and provide protection against **harmful radiations**.
- Due to the presence of OH group, flavonoids are partially soluble in water, however, when they form a bond with glucose they are known as glycosides, which increase their solubility, and they are stored in **vacuoles**.
- They are responsible for colors of flowers of white daisies, narcissus, off-white and yellow *chrysanthemum* cape sorrels. **Quercetin** is common in many fruits and vegetables.
- **Anthocyanins** are sugar-containing flavonoids stored in plant vacuoles having many OH groups. Their polar nature increases their solubility in water. They form blue, pink, purple and red shades in the organs where they are synthesized (**Figure 5**). However, depending upon their location in plants and the time at which genes for anthocyanins formation are expressed determines their role in plants. In *Hydrangea* and *Petunia* flowers, shades of anthocyanins are determined by soil pH (Enaru *et al*., 2021).

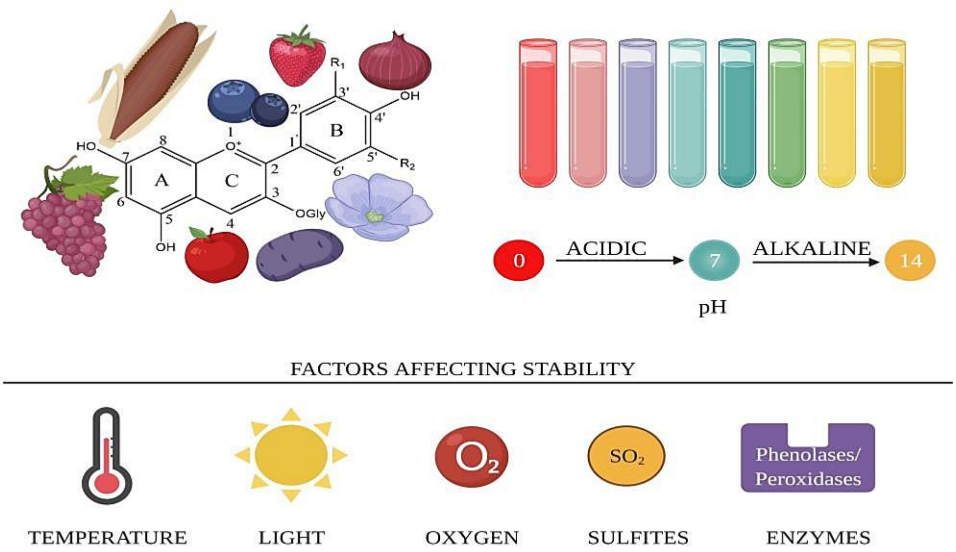

Figure 5. Anthocyanins: factors affecting their stability and degradation (Khan, 2017)

- Flavanoids find numerous applications in many industries like food, pharmaceutical, cosmetic, nutraceutical industries as well as in floriculture. Flavonoids and polyphenols consumption prevents oxidative stress that may cause change in lipid peroxidation in arterial macrophages and in lipoproteins and therefore, beneficial for reducing cardiovascular and inflammatory diseases as well as others.

Biotechnology of Flavonoids

● Flavonoids biosynthetic pathways are genetically engineered to produce novel flower colors in spp. of petunia, gerbera, rose, carnation, lisianthus and torenia.

● Other objectives of metabolic engineering of flavonoids pathways are to produce disease-resistant crops. Genes for enzymes like stilbene synthase from grapes are inserted in wheat and barley to produce transgenic plants resistant to fungal attack.

● *Anthocyanins* and *betalains*[18] can also be used to make dye-sensitized solar cells due to their ability to convert solar energy in chemical energy (Calogero *et al.*, 2012). *Betalains* are used as food and drink additives in meat, and in beverages.

[18] Betalains are a class of red and yellow tyrosine-derived pigments found in plants of the order *Caryophyllales* where they replace anthocyanin pigments.

2.4. Glycosides are Sugar-Containing Natural Products

Glycosides are sugar-containing secondary metabolites attached with a non-sugar molecule and provide defense to organs where they are synthesized. They can be found as ***cardioactive glycosides*** or ***cyanogenic glycosides,*** which release hydrogen cyanide (HCN) due to breakdown of cell walls, and also as ***steroid glycosides*** or ***saponins***, which form a soapy mixture when mixed with water.

- ***Cardioactive glycosides*** are found in plants like *purple foxglove* (*Plantaginaceae*), *Nerium oleander* (*Apocynaceae*) and are defensive against animals. They are abundant in leaves, stem bark and seeds of plants of the family ***Rosaceae*** (Plants like peach, almond, cherries, and passion fruits).
- There are more than **2600** cyanogenic glycosides reported in **110 families** of different plants. Many of these are found in cereals like wheat, rye, barley, maize, oats, sugar cane, millets and sorghum. **Amygdalin** from bitter almonds, **linamarin** from linseed[19], **manihotoxin** from *Manihot esculenta*[20] are common cyanogenic glycosides (see Box 1).
- ***Saponins*** are gylcosidic triterpenoids or steroids and are characteristics of mostly eudicots. They have antimicrobial, insecticidal and allelopathic activities.

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[19] *Linum usitatissimum* or common flax (*Linaceae*)
[20]*Euphorbiaceae*, some genera *Euphorbia*, *Ricinus*, Hevea.

Box 1

Some examples for the main functions of secondary metabolites in plants:

- **Chemical Defense:**

Many plants are filled with toxins that kill herbivores or, at the very least, deter their grazing behavior.

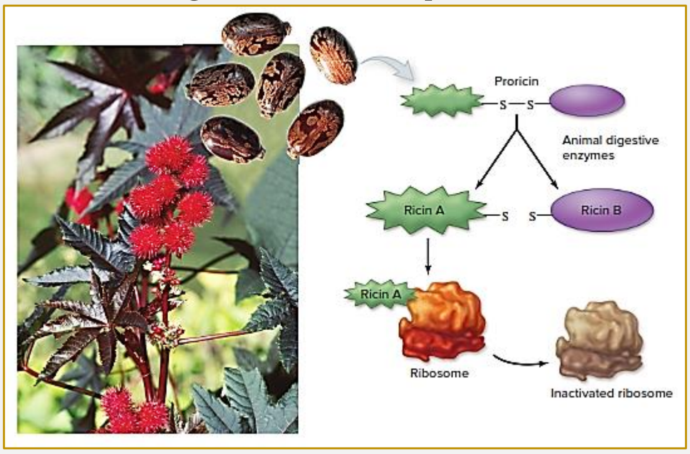
Exp1:

The production of cyanide (HCN). Over 3000 species of plants produce cyanide-containing compounds called ***cyanogenic glycosides*** that break down into cyanide when cells are damaged. Cyanide stops electron transport, blocking cellular respiration.

Cassava (genus ***Manihot***), a major food staple for many Africans, is filled with cyanogenic glycosides (specifically, manihotoxins) in the outer layers of the edible root. Unless these outer layers are scrubbed off, the cumulative effect of eating primarily cassava can be deadly. (Similar molecules: ***Amygdalin*** from *Rasaceae*).

Exp2.

Ricin, an alkaloid found in castor beans (*Ricinus communis*), is **six times more lethal than cyanide** and **twice as lethal as cobra venom.** A single seed from the plant, which is still grown in flower gardens, can kill a young child if ingested. Ricin is found in the endosperm of the seed as a heterodimer composed of ricin A and ricin B, joined by a single disulfide bond. This heterodimer (proricin) is nontoxic, but when the disulfide bond is broken in humans or other animals, ricin A targets the 28S rRNA of the ribosome. Death occurs because ricin A cleaves the adenine from nucleotide 4324 in the 28S ribosome, resulting in a neutral ribose that changes the structure of rRNA and thus inhibits translation. A single ricin A molecule can inactivate 1500 ribosomes per minute, blocking translation of proteins.



In 1978, Bulgarian expatriate and dissident *Georgi Markov* was about to board a bus in London on his way to work at the BBC when he felt a sharp stabbing pain in his thigh (**THī**). A man near him picked up an umbrella from the ground and hurriedly left. Markov had been injected via a mechanism in the umbrella tip with a pinhead-sized metal sphere containing 0.2 mg of ricin. Markov died four days later.

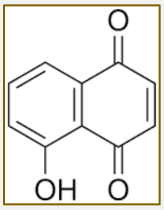
In addition to toxins that kill, plants can produce chemical compounds that make potential herbivores ill or that repel them with strong flavors or odors.

➢ **Plants can poison other plants (Allelopathy).**

Some chemical toxins protect plants from other plants. ***Allelopathy***[21] occurs when a chemical compound secreted by the roots of one-plant blocks the germination of nearby seeds or inhibits the growth of a neighboring plant.
This strategy minimizes shading and competition for nutrients, while it maximizes the ability of a plant to use radiant sunlight for photosynthesis.
Allelopathy works with both a plant's own species and different species.

Exp.

Black walnuts (*Juglans nigra* L.) are one of the best-known allelopathic plants. Very little vegetation will grow under a black walnut tree because of allelopathy. Roots of Black walnut trees naturally contain a chemical called ***juglone (5-hydroxyalphanapthaquinone)***, which can inhibit the growth of some plants.



Further reading:

1. Martinez, M., & Diaz, I. (2024). Plant Cyanogenic-Derived Metabolites and Herbivore Counter-Defences. Plants, 13(9), 1239.
2. Lamb, F. I. (1984). Castor bean lectins: construction and sequencing of cDNA clones (Doctoral dissertation, University of Warwick).
3. Funt, R. C., & Martin, J. (1993). Black walnut toxicity to plants, humans and horses. Ohio State University extension fact sheet HYG-1148-93.
4. Macías, F. A., Durán, A. G., & Molinillo, J. M. (2020). Allelopathy: The chemical language of plants. Progress in the chemistry of organic natural products 112, 1-84.

[21] Allelopathy is a biological phenomenon where one plant inhibits the growth of another.

Box 2

Biological activities of some secondary metabolites

Major research efforts on plant secondary metabolites are in progress because of their potential benefits, as well as dangers, to human health.

- ***Soy and phytoestrogens***

Soybean products contain ***phytoestrogens***, compounds very similar to the human hormone estrogen. In soybean plants, ***genistein*** is one of the major phytoestrogens. Comparative studies between Asian populations that consume large amounts of soy foods and populations with lower dietary intake of soy products are raising interesting results. For example:

- Reducing the rate of **prostate cancer** in Asian males, which might be accounted for by the down-regulation of androgen and estrogen receptors by a phytoestrogen.
- Minimizing **menopausal symptoms** caused by declining estrogen levels in older women.

- ***Paclitaxel (Taxol) and breast cancer***

Paclitaxel (Taxol), a secondary metabolite found in the Pacific yew (*Taxus brevifolia*), is effective in fighting cancer, especially breast cancer. The discovery of the pharmaceutical value of paclitaxel (Taxol) created an environmental challenge. The very existence of the Pacific yew was being threatened as the shrubs were destroyed so that paclitaxel (Taxol) could be extracted.

Fortunately, organic chemists developed an effective and affordable strategy for synthesizing paclitaxel (Taxol) in the laboratory. Paclitaxel (Taxol) is not an isolated case of drug discovery in plants.

The hidden pharmaceutical value of many plants may lead to increased conservation efforts to protect plants that have the potential to contribute toward human health.

Although the plant pharmaceutical industry is growing, it is certainly not a new field. Until recent times, almost all medicines used by humans came from plants.

- ***Quinine and malaria***

In the 1600s, the Incas of Peru were treating malaria with a drink made from the bark of ***Cinchona*** trees. Malaria is caused by four types of human malaria parasites in the genus ***Plasmodium,*** which are carried by female ***Anopheles*** mosquitoes. ***Plasmodium falciparum*** is the most lethal of the four types. Symptoms include severe fevers and vomiting. The parasite infects red blood cells, and death can result from anemia or blocking of blood flow to the brain.

By 1820, the active ingredient in the bark of ***Cinchona*** trees, **quinine,** had been identified. In 1944, American chemists Robert Woodward and William Doering synthesized quinine. Now several other synthetic drugs are available to treat malaria.

Exactly how quinine and synthetic versions of this drug family work has puzzled researchers for a long time. Quinine can affect DNA replication, and when ***P. falciparum*** breaks down hemoglobin from red blood cells in its digestive vacuole, an intermediary toxic form of heme is released. Quinine may interfere with the subsequent polymerization of these hemes, leading to a buildup of toxic hemes that poison the parasite.

Unfortunately, even today malaria is a major threat to human health, causing over 500,000 deaths per year. Ninety percent of these deaths occur in sub-Saharan Africa. Worldwide, an estimated 198 million individuals are infected. ***P. falciparum*** strains have acquired resistance to synthetic drugs, and quinine is once again the drug of choice in some cases.

Ending remark

Herbal remedies have been used for centuries in most cultures. A resurgence of interest in plant-based remedies is resulting in a growing and unregulated industry.

Although herbal remedies have great promise, we need to be aware that each plant contains many secondary metabolites, many of which can cause harm to herbivores, including humans.

Problems Chapter 2

MCQ: Select the best answer from options given below:

1. __________ is generally used in the prevention of heart attacks, strokes, fever and pain.
   a) Quinine
   b) Morphine
   c) Aspirin
   d) VICKS
2. Commercially important resins produced by many plants in their resin canals are chemically:
   a) Alkaloids
   b) Tterpenoids
   c) Glycosides
   d) Phenols

3. Plants produce different pigments which are generally stored in vacuoles, however, ____ pigment is not stored in plant vacuoles.
   a) Anthocyanin
   b) Flavonoids
   c) Carotenoids
   d) Chlorophyll
4. Many polyterpenoids which are produced in plants are responsible for giving _______ to plants.
   a) Pigments
   b) Odor
   c) Resin
   d) Caffeine
5. One of the secondary metabolites which is natural constituent of wood and produced through shikimic acid pathway is __________.
   a) Terpenoids
   b) Phenols
   c) Saponins
   d) Alkaloids
6. Amygdalin, a cyanogenic glycoside is used in many drugs and exists naturally in the seeds, pits and bark of many plants of family __________.
   a) Fabaceae
   b) Rosaceae
   c) Solanaceae
   d) Rubiaceae

Open-ended question (OEQ):

1. Distinguish between primary and secondary metabolism.
2. What are terpenes? How do they originate within the plant and what functions do they serve?
3. Explain why secondary metabolites, originally designed by plants to deter pests reattract pollinators, have commercial value to humans.
4. Explain why sterols may be considered both primary and secondary metabolites.
5. What do saponins, cardenolides, and amygdalin have in common?
6. Why are amino acids such as phenylalanine and tryptophan essential in the human diet?
7. Alkaloids are -for the most part- chemically unrelated. What is the basis for grouping them together?
8. In what ways do plants have some control over insect herbivory?
9. How do plants respond to invasion by microbial pathogens?

CHAPTER III

Valorization of plant substances

CHAPTER III

Valorization of plant substances

Learning Outcomes:

By the end of this chapter, students should be able to:

- Recognize key terms related to the valorization of plant extracts (Secondary metabolites).
- Explain the main industrial sectors that utilize plant extracts for their valorization.
- Distinguish between standardized and unstandardized medicines.
- Provide examples of plant materials with potential value and describe methods of their valorization.

Introduction

Plant extracts are vital sources of various biologically active compounds, such as phytosterols, amino acids, lipids, phenols, flavonoids, anthocyanins, alkaloids, and terpenoids. These compounds exhibit a range of beneficial biological activities, making them valuable in numerous applications. Due to their antioxidant, anti-inflammatory, antidiabetic, antibacterial, antifungal, and anticancer properties, the use of plant extracts for therapeutic and prophylactic purposes, as well as in cosmetic formulations, has significantly increased over the past few decades Moreover, plant extracts play a crucial role in pest and weed control, contributing to food security, environmental protection, and human health (Ning *et al*., 2021). Consequently, plant extracts are becoming increasingly important in fields such as medicine, agriculture, and environmental protection.

According to the *World Health Organization* (WHO) "***Medicinal and aromatic plants***" (MAPs) can be defined as '***plant-derived materials or products with therapeutic or other human benefits, which contain either raw or processed ingredients from one or more plants'*** (WHO, 2001). Other specific terms used for medicinal and aromatic plants are (American Botanical Council, 2019):

a) "**herb or culinary herb**" refers to any aromatic plant material from temperate regions, used in minor quantities to flavor foods and beverages, but has little or no known nutritional value,

b) "**spice**" implies to an aromatic plant material from tropical regions used in minor quantities to flavor foods and beverages, but has little or no known nutritional value,

c) "**medicinal plant**" describes various plants used for treatment of disease or other body afflictions,

d) "**essential oil**" indicates that a volatile oil can be extracted from plants by distillation, solvents or expression,

e) "**poisonous plant**" indicates to plants containing alkaloids or other substances that may produce toxic effects when introduced into the body.

What is the difference between medicinal and aromatic plants?

- Medicinal plants, also known as medicinal herbs, can be defined as plants that possess therapeutic properties or exert beneficial pharmacological effects on the human or animal body.
- Aromatic plants provide products that are extensively used as spices, flavoring agents and in perfumes and medicine. In addition, they also provide raw materials for the production of many important industrial chemicals (e.g., dyes).

MAPs are a set of plants used in *food processing* and *herbal medicine (phytotherapy)* for their secondary metabolites/bioactive molecules (e.g., *alkaloids*, *aromas, essential oils*).
They are part of the ingredients of traditional medicine or alternative medicine, and in the form of extracts, are used in *aromatherapy.*

Phytotherapy:

Phytotherapy is one, if not the oldest method, used in the treatment of infectious diseases.

Aromatherapy: is one branches of **phytotherapy**, which uses aromatic plant extracts (essences and essential oils). It differs from herbal medicine, which uses all the elements contained in the plant.

Historical overview of MAPs uses!

MAPs have always played an important socio-economic and environmental role in the history of peoples throughout the ages and civilizations. For example, archaeological studies, carried out in the 1970s, found the remains of a human body in northern Iraq dating back to the prehistoric period (more precisely the Neanderthal around 60,000 years ago) surrounded by medicinal plants (Box 3).

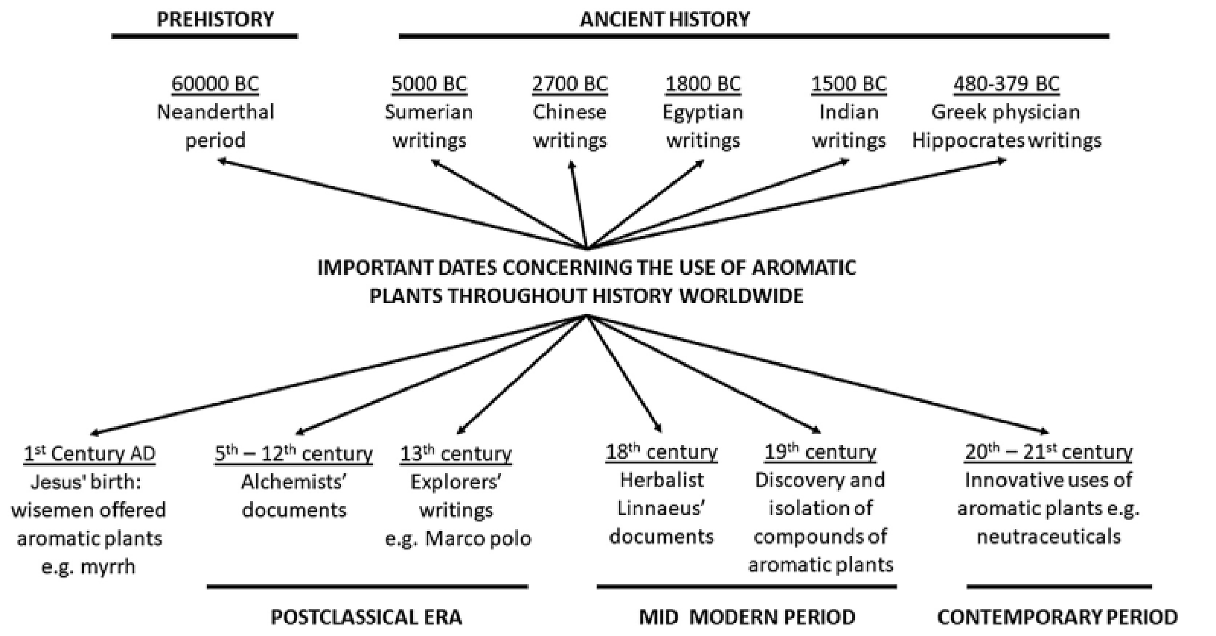
The analysis of manuscripts and other objects (clay tablets, etc.), has shown that, throughout human history going back in time, all human civilizations (Sumerians 5000 BC, Chinese 2700 BC, Egyptians 1800 BC, Indians 1500 BC, etc.) were marked by the use of MAPs (for culinary or medical needs).

Similarly, from the first century to the 18th century, the use of plants in medicine experienced a clear progression, particularly in the era of Muslim civilization. In addition to the translation into Arabic language of the great writings of the Greeks (Hippocrates, Dioscorides and Galen), many Islamic naturalists, botanists and doctors (Abu Bakr Arazi, Ibn Sina, Ibn al-baytar, etc) have contributed to the progress of our knowledge of MAPs use (Box 3).

Conversely, from the 2nd World War, and the discovery of synthetic drugs (aspirin, penicillin, etc.) the use of MAPs began to decline to give way to the modern medicine as well as the use of synthetic chemicals in medicine. Nevertheless, the development of the resistance of microorganisms, the appearance of side effects of synthetic medicines, the harmful effects of food additives on health and the environment have given reason for the spectacular return to the use of aromatic and medicinal plants to develop new products that are more effective and risk-free for health and the environment (Abdallah *et al*., 2023).

Box 3

The use of MAPs in BCE (Before Christ Era) and CE (Common Era) periods worldwide.



Prehistory:
It appears that the very first evidence of humans using plants occurred in a **Neanderthal** flower burial site in **Northern Iraq**, approximately **60,000** years BC, as the remains of a body were found surrounded by at least seven medicinal plants, including *Ephedra* (Solecki & Shanidar, 1975; Jamshidi-Kia *et al.*, 2018).

Ancient history:
Based on historical reviews:

- Sumerian writings (5000 BC).

Ex. several written formulas on clay stones were discovered around the Nagpur area, 5000 years BC. These formulas involved 12 plant preparations, based on more than 200 herbs, including common alkaloids such as poppy, henbane, licorice, and mandrake (Duke, 2002; Kelly, 2009).

- Chinese writings (5000-2700 BC) – Chinese *Materia Medica* (1100) listed over 300 plants.
- Egyptian writings (3000-1800 BC) – "Ebers Papyrus" manuscripts, back in around 1800 BC, a good knowledge of plant species as medicines had been exhibited (Jones, 1996). Specifically, there was a collection of approximately 800 prescriptions, quoting of 700 plants, including garlic, onion, aloe, pomegranate, coriander, and juniper.
- Indian writings (1500 BC) – The holy books of Atharva and Rig Veda (Buddhism), Ayurveda (the science of life),
- Greek writings (500 BC) – Hippocrates (Father of Medicine), Theophrastus (Father of Botania)

Common Era:

- 1st Century AC, Dioscorides writings (*De material de medica*), Glen writings (20-90 AC).
- 5th – 10th century, Alchemists (dominated by Dioscorides and Galen writings)
- 10h – 15th century, the Dioscorides and Galen writings were translated into the Arabic language and enhanced their knowledge and remedies (Razi, Ibn sina or Avicenna; Abou alabass Anabati; Ibn Albaytar, etc.)
- 16th -19th century (Herbalist Linnaeus' documents – discovery and isolation of compounds of aromatic plants)
- 20th – 21st century (Innovative uses of aromatic plants, e.g. neutraceuticals)

Let see now, how the valorization of MAPs can be done?

In fact, plant-derived products can be valorized by using them, at least, in three sectors of interest:

- **Agro-food industry**
- **Pharmaceutical industry**
- **Cosmetic industry**

Chapter III – Part 1
Valorization of Plant Substances in The Agri-Food industry

1. Introduction:

The agri-food (also agro-food) industry can be defined as ***the sector, which includes all operations related to processing, preserving, preparing, and packaging agricultural and food products carried out in industrial production units***. Therefore, the agro-food industry was concerned about producing many products such as plant and animal food products, fertilizers, pesticides, etc. However, in this course, we will focus on food products.

Currently, the world suffers from numerous health and environmental problems that are tightly linked to the use of chemicals in food and agriculture. However, these problems can be fixed or at least prevented using MAP phytochemicals.

Therefore, different processes can help in the valorization of plant products and, particularly, MAP products, among others:

- Prevention of chronic diseases and nutritional deficiencies by food or dietary supplements.
- Improvement of food products quality by natural condiments (flavors)
- Production of natural food colorants (additives).
- Food preservation (shelf-life improvement) using natural antioxidants and antibiotics.
- Production of edible packaging
- Etc.

2. Phytonutrients

Phytonutrients are ***natural compounds found in foods of plant origin***. Acting in combination with other essential nutrients, they have health benefits (Derbel & Ghedira, 2005). They have several biological and pharmacological properties explaining their benefits on human health. Some of them are active through their antioxidant power; others participate in the enzymatic detoxification of carcinogenic substances present in the body.

Due to the diversity of their biological and pharmacological properties, phytonutrients are of interest in the ***prophylaxis*** (prevention) of various diseases, in particular *cancer*, *cardiovascular*, *ophthalmic* and *inflammatory diseases* (See for review, Derbel & Ghendira, 2005).

Current evidence supports the use of phytonutrients as therapeutic agents against a wide variety of modern lifestyle disorders. However, their efficacy is limited by their low bioavailability (See for review Kan *et al*., 2022).

Common phytonutrients include polyphenols, phytosterols, saponins, and carotenoids. Well-documented phytonutrients, such as catechins, curcumins, anthocyanins, quercetin and chlorogenic acid, can be easily ingested from diets, and many studies have demonstrated their role as anti-cancers, neuroprotectors, anti-aging, as well as in the treatment of metabolic disorders, and other diseases (Socala *et al*., 2020; Baby *et al*., 2018).

A. Carotenoids:

Carotenoids (plant pigments) are very well known and widely studied phytonutrients. There are over 700 carotenoids, but only about 50 of them are found in our food and absorbed by the human body. The best known of the carotenoids is beta-carotene, which is a precursor of vitamin A. It is responsible for the orange color of carrots, melon, mango, but also for the green color of certain leafy vegetables (**Table 3**).

We also find in this vast family, ***lycopene*** that colors tomatoes red, pink grapefruit and watermelons, ***lutein*** that lends its pigment to ***dark green vegetables*** (green cabbage, spinach, broccoli…), ***zeaxanthin*** responsible for the yolk color corn and ***astaxanthin***, a pink pigment extracted from the shells of crustaceans or certain algae. Carotenoids are fat-soluble substances (linked to fats) with high antioxidant capacity.

Food sources:

Table 3. Common dietary sources of carotenoids in regular vegetable foods (µg/100g fresh weight) according to Jaswir *et al.* (2011).

Food	B-carotene	Lutein	B-Cryptoxanthin	Lycopene	Zeaxanthin
Carrot	7975	271	-	-	-
Watercress	5919	10713	-	-	-
Spinach	4489	6265	10		466
Broccoli	1580	2560	-	-	-
Lettuce	890	1250	-	-	-
Green peas	548	1840	-	-	-
Red pepper	1700	270	250		600
Tomato	608	77		4375	9
Orange	250	120	700		
Mandarin	275	50	1775		140
Apricot	3500	70	120	-	-
Mango	3100	-	800	-	-
Papaya	640		700	3400	-
Watermelon	180	20	300	4750	-

After the U.S. DEPARTMENT OF AGRICULTURE (https://fdc.nal.usda.gov/).

B. Polyphenols

Polyphenols allow the plant to protect itself against attacks, such as infections and ultraviolet rays. They are the most abundant antioxidant substances in our diet. Humans ingest or absorb with food about **1 gram** of phenolic compounds each day, i.e. 10 times more than vitamin C and 100 times more than carotenoids and Vit E.

Research on the health effects of phenolic compounds or polyphenols began later than for other antioxidants.

Food sources:

Polyphenols are found in great abundance in our diet. The richest sources, all polyphenols combined, are leafy vegetables, fruits, green tea and red grapes etc. (See for review Manach *et al*., 2004).

Table 4. Nutritional distribution of certain flavonoids.

Fruits - Vegetables	Arabic noun	mg/Kg	Major Molecule
Parsley	بَقدونس	500	Apigenin
Lettuce	خَسّ	320	Quercetin
Onion	بصل	300	Quercetin + Kaempferol
Celery	كرفس	100	Apigenin+ luteolin
Chive	الثوم المعمر (سيبولات)	100	Quercetin + Kaempferol
Leek	كراث	100	Quercetin + Kaempferol
Beans	فول	70	Quercetin + Kaempferol
Broccoli	بروكلي	35	Quercetin + Kaempferol
Tomato	طماطم	10	Quercetin + Kaempferol
Cauliflower	قرنبيط (شفلور)	3	Quercetin + Kaempferol
Potato	البطاطس	3	Quercetin + Kaempferol
Orange, lemon	البرتقال و الليمون	170/280	Hesperitin
Grapefruit	جريب فروت (بمبليموس)	270/600	Naringenin
Grapes	العنب	50 /100	Myricetin + Quercetin
Apricots	المشمش	55	Quercetin
Blackberries	توت	50	Quercetin + Kaempferol
Apples	تفاح	30	Quercetin
Raspberries	توت العليق	30	Quercetin + Kaempferol
Plums	برقوق	30	Quercetin + Kaempferol

Some examples on the valorization of plant products as phytonutrients:

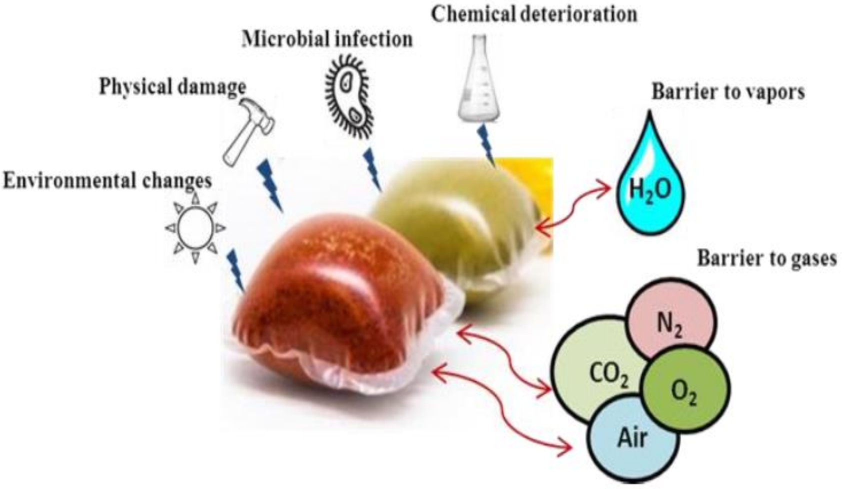
Example N°1.

❖ This article deals with the impact of plant extracts and fruit juices as a natural source of antioxidants on the production of yoghurts (Gad *et al*., 2015).

It is believed that the consumption of antioxidants provides protection against oxidative damage and helps improving the health status of the consumer.
Incorporating natural antioxidant compounds into yogurt is a simple way to improve public health and/or reduce disease risk. Carrot and cantaloupe juice were used to form the structure of yogurt with different concentrations.
Sensory characteristics, carotenoid content, β-carotene content and antioxidant activity (DPPH and FRAP) were studied during refrigerated storage at 5°C for 12 days.
The total carotenoid content in all yogurt samples gradually decreased along cold storage. There was a sharp decline in β-carotene of yogurt after cold storage for 6 and 12 days whereas yogurt retained its antioxidant activity during storage.
Yogurt made from 8% carrot juice was the best form of fermented dairy product to deliver the healthy benefits of carotenoids to the consumer.

Example N°2.

❖ This article deals with the use of aromatic plants for the production of edible packaging more efficient and more beneficial to human health (Ravi *et al*., 2020).



Function and usefulness of edible film and coatings in the food industry

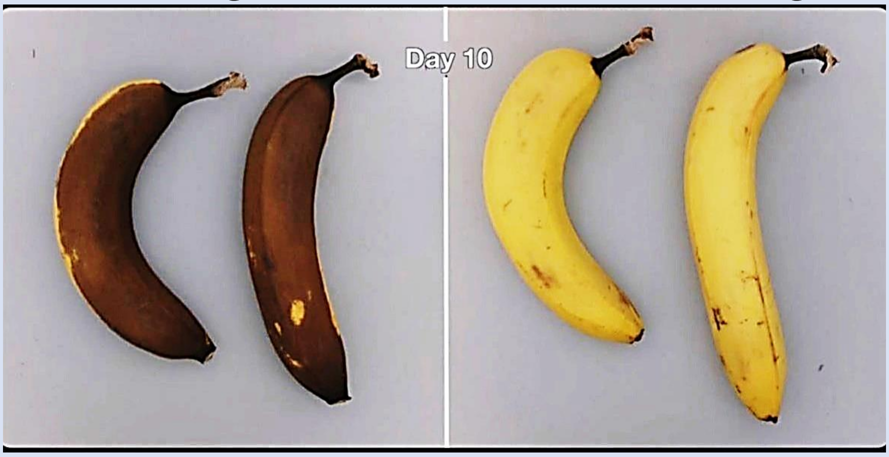
In this article the authors discuss the question of the use of extracts and/or essential oils of aromatic plants (spices in particular) to produce more efficient edible packaging in addition to having various beneficial effects on the health of the consumer.
Edible packaging[1] has received particular attention in recent years. The main advantage of these being respect for the environment (counter to plastic pollution). The basic material used in edible packaging (lipids, polysaccharides, proteins) is generally recognized as safe and acts as a barrier against gases, light, temperature, humidity, microbial infection, etc.

[1] Edible food packaging is a type of packaging that is designed to be eaten or has the ability to biodegrade efficiently like the food that it contains.

Due to their richness in bioactive compounds known for their antioxidant and antimicrobial properties, the incorporation of spice extracts or their essential oils in edible packaging will exert antimicrobial activity against foodborne pathogens thus preventing food spoilage, improves the shelf life and also increases the nutritional value of the final product.
As for their antioxidant properties, they will delay the oxidation of lipids. Food allergies and intolerance associated with packaging materials will be reduced by incorporating bioactive substances from aromatic plants in the manufacture of edible packaging.
There are different types of edible packaging (for more details see the coating processes on the following web pages: http://genie-alimentaire.com/spip.php?article329, and the following videos).

EDIPEEL

An edible coating that increases the life of fruits and vegetables



Edipeel is an edible, non-chemical coating that increases the shelf life of fruits and vegetables. It is designed specifically for each type of fruit and vegetable. *Edipeel* comes from food "waste", such as orange or grape peels, from which a puree is produced.
Different molecules are extracted from this mash, transformed into powder and sent directly to the farms. The powder, transformed back into liquid, is sprayed on fruits and vegetables during the cleaning cycle. When *Edipeel* dries on the food, a residual layer forms there. This protective layer reduces water loss and oxidation of the fruit or vegetable, thus increasing its lifespan by 3 to 5 times, without refrigeration.
Edipeel could turn out to be a revolutionary product in the preservation and transport of fruits and vegetables. Indeed, for maximum shelf life, these foods are currently transported in refrigerated trucks that use a lot of energy and incur significant costs. With *Edipeel,* the mode of transport could require less refrigeration. By increasing the shelf life of food, *Edipeel* therefore benefits farmers as much as grocers and restaurateurs, as it reduces the waste of food products.
The company *Apeel Sciences* is also working on a new product that would repel insects without chemical input.

Another example: The use of nutraceutical products!

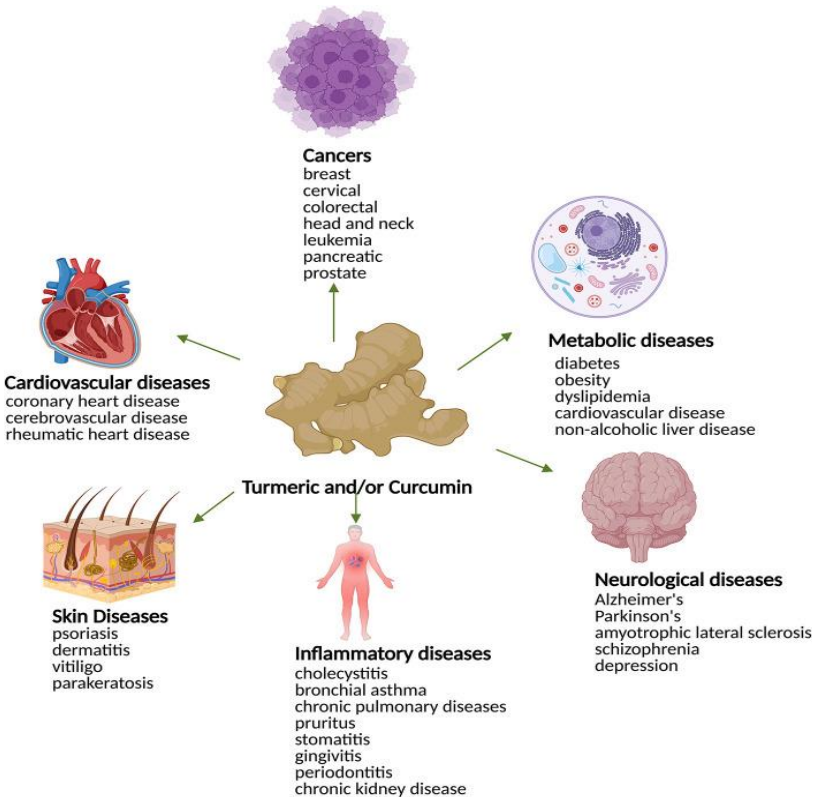
The use of turmeric or curcumin to fight against chronic diseases!

Many studies show that the ***golden spice*** has enormous health and medicinal benefits for humans. For example, in a review, the authors have analyzed more than 20.000 papers in order to find out if **Turmeric** and **curcumin** have really a role to play in fighting against cancers (Kunnumakkara *et al*., 2023).

Dietary supplement

Turmeric Curcumin with Black Pepper Extract 1500mg - High Absorption Ultra Potent Turmeric Supplement with 95% Curcuminoids and BioPerine - Non GMO https://www.amazon.com/Turmeric-Bioperine-Available-Standardized-Curcuminoids/dp/B01DBTFO98

Diverse function of turmeric and/or curcumin against diseases. This figure shows the diseases against which turmeric and/or curcumin are shown to be effective (Kunnumakkara *et al*., 2023).



CHAPTER III – Part 2

Valorization of Plant Substances in Pharmaceutical industry

1. Introduction:

Since time immemorial natural herbs were extensively used for the treatment and prevention of various ailments and in past few decades, due to extensive research in traditional system of medicine various herbal medicines have been developed for the prevention and treatment of diseases, which are environmentally, organically safe and inexpensive (Saggar *et al*., 2022).

Plants are an inexhaustible natural reservoir for the world's **pharmacopoeias**[2]. Several important medicines are made from active substances of plant origin. In addition, many modern medicines have been made from these raw materials.

MAPs are used in divers' manners: directly as fresh, dry, processed, stabilized, extracted form or formulated with other plants or synthetic excipients.
In all cases, the herbal material used to manufacture the drug (medicine) form must be checked for its:

- *Efficacy,*
- *Safety,*
- *Quality, (meets the requirements of EMA[3] or FDA[4], etc.).*

To this end, the World Health Organization (WHO) has made available to Member States guides and standards to harmonize and secure their use. Thus, many African countries have adopted these tools after appropriate modifications to advance research and development (R&D) of plant-based drugs.

About a **quarter** (1/4) of all EMA and/or FDA approved drugs are plant based, with well-known drugs such as ***Paclitaxel***[5], ***Morphine***[6], ***Vinblastine***[7]***, Quinine*** and ***Artemisinin***[8] having been isolated from plants (**Figure 6**). In the face of global public health challenges, natural

2- or *drug-making*.
3- European Medical Agency
4- Food and Drug Administration
5- anticancer
6- narcotic analgesic, tranquilizer, isolated by *Serturner* in 1806.
7- anticancer
8- antimalarial

products research and development (R&D) potentially plays a pivotal role in innovative drug discovery (Thomford *et al*., 2018).

Traditionally, plant extracts are used as concoctions made of combinations of different ingredients. Individually some of the ingredients do not have therapeutic activities, but require their synergistic activities (Kiyohara *et al*., 2018).

Current challenges to the use of natural products and difficulty in accepting their therapeutic efficacy include:

1) *lack of standardization procedures,*
2) *lack of isolation of pure chemical products or compounds,*
3) *lack of elucidation of biological mechanisms*
4) *documented clinical trials according to "standards".*

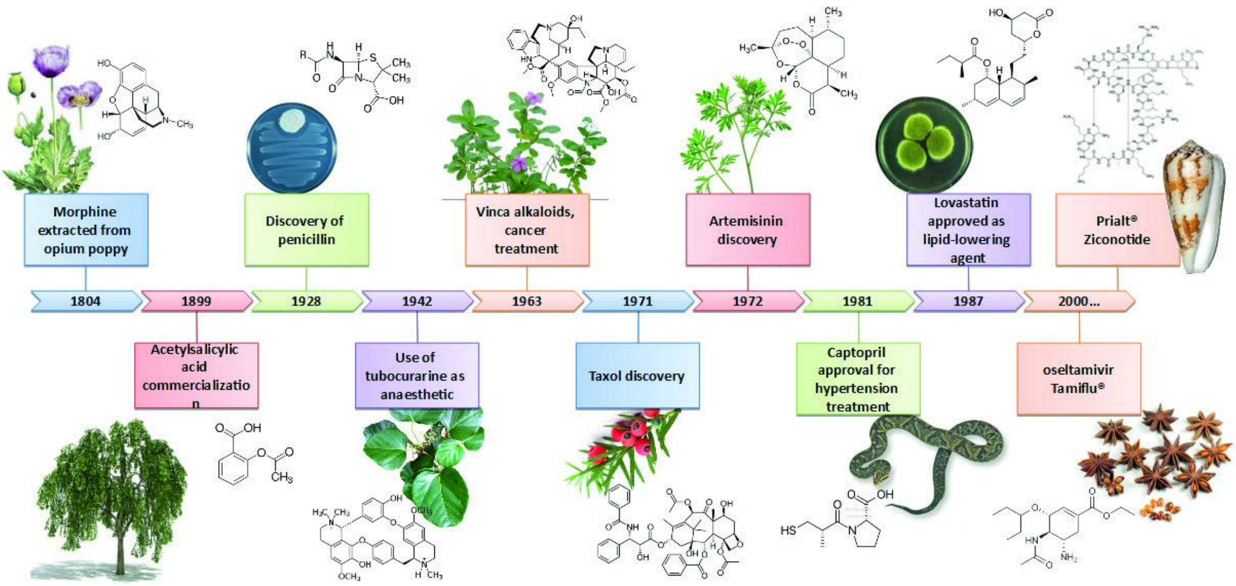


Figure 6. A few natural products used as medicines identified in the last century (Valli, & Bolzani, 2019).

2. Herbal Drug Standardization and Evaluation

In recent years, there has been a great demand for **plant-derived products** in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics (Ogbonna *et al*., 2019).

There are thousands of herbal-drug manufacturers around the world. The majority of them are located in China and India. Due to a lack of infrastructure, skilled manpower, reliable methods, and stringent regulatory laws, most of these manufacturers produce their products on a very tentative basis (Patel *et al*., 2006a).

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis (Patel *et al*., 2006b).

"**Standardization**" (Normalisation in French, التوحيد القياسي in Arabic) expression *is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also means adjusting the herbal-drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity respectively by adding excipients or by mixing herbal drugs or herbal drug preparations* (Bhutani, 2003).

"**Evaluation**" of a drug means *confirmation of its identity and determination of its quality and purity and detection of its nature of adulteration* (Kokate *et al*., 2005).

Standardization of herbal drugs is not an easy task as numerous factors influence the ***bioefficacy*** and ***reproducible*** therapeutic effect. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in the case of polyherbal drugs (Patel *et al*., 2006).

The Standardization of crude drug materials includes the following steps:

Authentication[9]: Each step has to be authenticated.

a) Stage of collection.

b) Parts of the collected plant.

c) Regional status.

d) Botanical identity like phytomorphology, microscopically and histologically analysis (characteristic of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibers, vessels etc.) (Shrikumar *et al*., 2006).

Quality control:

The herbal formulation, in general, can be standardized schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed.

The preparations with better clinical efficacy are to be selected. After all the routine physical, chemical and pharmacological parameters are to be checked for all the batches to select the final finished product and to validate the completely manufacturing process (Shrikumar *et al*., 2006).

[9] the process or action of proving or showing something to be true, genuine, or valid.

The ***stability*** parameters for the herbal formulations, which include physical, chemical and microbiological parameters, are as follows:

- Physical parameters include color, odor, appearance, clarity, viscosity, moisture content, pH, disintegration time, friability, hardness, flow ability, flocculation, sedimentation, settling rate and ash values.
- The chemical parameters include chemical tests, chemical assays, and instrumental analysis. This evaluation should be done according to standard methodologies (ISO[10], AFNOR[11], BS[12], ASTM[13], DIN[14], etc.)
- **Chromatographic analysis** of herbals can be done using TLC, HPLC, HPTLC, GC, UV, GC-MS and fluorimetry, etc.
- **Microbiological parameters** include total viable content, total mold count, etc. Limiters can be utilized as a quantitative or semi-quantitative tool to ascertain and control a number of impurities like the reagents used during abstraction of various herbs, impurities coming directly from the manufacturing vessels and from the solvents, etc.

3. Guidelines for Herbal Drug Standardization

3.1. WHO Guidelines

The subject of herbal drug standardization is massively wide and deep. The guidelines set by WHO can be summarized as follows:

1. Reference to the identity of the drug. Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements, etc.
2. Refers to the physicochemical character of the drug. Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal assays, quantitative estimation protocols, etc.
3. A reference to the pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index, etc.
4. Toxicity details- pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like *E. coli, Salmonella, P. aeruginosa, S. aureus, Enterobacteria,* etc.
5. Radioactive contamination.

[10] International Standards Organization
[11] Association française de normalisation
[12] British Standards
[13] American Society for Testing and Materials
[14] Deutsches Institut für Normung

The drug development process

Step 1: Discovery and Development.

Step 2: Preclinical Research.

Step 3: Clinical Research.

Step 4: Regulatory Drug Review.

Step 5: Post-Market Drug Safety Monitoring.

For more explanation see the following document:

https://www.nebiolab.com/drug-discovery-and-development-process/

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function.

Even when the chemical composition of a plant extract is known, the pharmacologically active moiety may not be. Environment, climate and growth conditions influence the composition, as does the specific part of the plant and its maturity. Monographs detailing standardization of active ingredients would improve the marketplace. Even if an herbal product is standardized to, for example, 4% of a constituent, the remaining 96% of ingredients is not standardized and may affect the product's solubility, bioavailability, stability, efficacy and toxicity. Just as controlled trials are necessary to establish safety and efficacy, manufacturing standards are required to ensure product quality (Boullata & Nace, 2000).

Nowadays newer and advanced methods are available for the standardization of herbal drugs like fluorescence quenching, the combination of chromatographic and spectrophotometric methods, biological assays, use of biomarkers in fingerprinting etc. Bioassay can play an important role in the standardization of herbal drugs and can also become an important quality control method as well as for proper stability testing of the product (Bhutani, 2003).

For more information see the following videos.

Plant for the production of solid dosage forms of pharmaceuticals

Half of the global market for active pharmaceutical ingredients is controlled by China.

The Birth of the Pharmaceutical Industry

Chapter III – Part 3
Valorization of Plant Substances in The Cosmetic Industry

1. Introduction:

The cosmetics industry is currently undergoing a significant transformation driven by a growing consumer demand for natural and sustainable ingredients (Kiese *et al*., 2024).

The use of plants as a source of active principles for cosmetics has significantly increased in the last few years. Nowadays, more and more plants are used in the formulation of products intended to improve physical appearance (care for the skin, hair, body, face, teeth, etc.) (Mansoor *et al*., 2023).

2. Medicinal plant ingredients used in cosmetic products

In recent years, there has been a significant surge in consumer awareness regarding the ingredients used in cosmetic products, leading to a growing demand for clean, sustainable, and natural alternatives (Kiese *et al*., 2024). Therefore, more and more raw materials of plant origin are present in current cosmetic formulas. They are common ingredients, such as:

- oils (palm, coconut, corn, jojoba), kinds of butter (apricot, shea), vegetable fats and waxes (lime);
- alcohols and fatty acids (lauric acid, palmitic acid, for example);
- gelling agents[15] such as guar flour, carob kernel flour, gum arabic, agar-agar, algins and potato, rice or wheat starch;
- natural surfactants[16] such as lauryl sulfate and disodium-laureth-sulfate from coconut oil or palm oil, betaine (a component of coconut butter);
- Sesame or wheat germ unsaponifiables.
- Etc.

3. Vegetable oils:

Many vegetable oils are natural care bases, to be used alone or in combination with essential oils.

Examples:

a. ***Sweet almond oil*** is very suitable for sensitive skin, especially that of babies. Rich in vitamin A, D and E, it is used to moisturize hands and delicate skin, and relieve itching.

[15] Additive intended to give the consistency of a gel to a preparation.
[16] a substance that tends to reduce the surface tension of a liquid in which it is dissolved.

b. ***Argan oil***, rich in vitamin E and polyunsaturated fatty acids, would slow down the drying of the skin. It is proposed to fight against chapping, small burns, stretch marks and to treat dry hair.

c. ***Borage oil*** is rich in polyunsaturated fatty acids and vitamins A, D, E and K, but its penetrating power is weak.

4. Thickeners and gelling agents:

They are hydrophilic macromolecules of semi-synthetic or synthetic natural origin capable of forming gels by trapping a large quantity of solvents (generally water).

In small quantities, they are thickeners. In general, they provide consistency to cosmetic products by improving their stability (e.g., shampoos, soaps, toothpastes, etc.).

Examples:

- Pectins
- Carob gum
- Guar gum (thickener powder made from legumes).
- Starch: Obtained from seeds or pips of fruits or vegetables. These are polysaccharides (sugar molecules)
- Arabic gum (*Meska horra*), acacia gum, or mastic gum.
- Aloe gel (*Xanthan gum*): Natural polysaccharide obtained from aloe leaves.

5. Surfactants:

These are SLMI (sodium lauroyl methyl isethionate) and SCI (sodium cocoyl isethionate), which are both surfactants derived from coconut and very often used in natural and organic cosmetics for their cleansing properties.

Other examples:

- Xanthan gum
- Tragacanth gum
- Rice starch powder
- Corn starch powder
- Cassava starch powder

6. Unsaponifiables:

Vegetable oils are mainly made up of fatty acids and to a lesser extent unsaponifiables. However, it is in this unsaponifiable part that a large part of the active agents is found, giving them excellent properties, which esterified, hydrogenated or mineral oils do not have.

7. Other active substances of plant origin:

Plant-based preparations, such as floral waters (obtained by the hydro-distillation of flowers such us witch hazel, rose, orange blossom, blueberry), flower infusions, or essential oils, enhance the formulations of numerous cosmetics due to their beneficial properties for the skin (Aguiar *et al*., 2022).

Witch hazel is well-known for its vascular protective properties and is commonly included in facial products for redness-prone skin.

Additionally, extracts from ginseng, arnica, chestnut (*Castanea sativa*), marigold (calendula), mallow (*Malva* sp.), climbing ivy, sage, chamomile (*Chamaemelum nobile*), agrimony (*Agrimonia eupatoria*), and sweet clover are all active plant-derived substances frequently used in cosmetic care.

Examples about the manufacturing of herbal soaps, creams, etc., (Analysis of Scientific paper):

A- Manufacturing an Herbal Soaps

Many cleaning and lubricating products utilize soap, which is a salt of a fatty acid. Soap is typically used as a surfactant in washing, bathing, and other housekeeping tasks, helping to remove dirt, dust mites, and unpleasant odors from the body. Herbal soaps, made primarily from plant parts such as seeds, rhizomes, and roots, offer additional benefits including antibacterial, anti-aging, antioxidant, and antiseptic properties. Nuts and pulps from these plants can be used to treat illnesses, injuries, or to enhance overall health (Arun, 2023).

Herbal soap is free of synthetic dyes, flavors, fluorides, and other additives, when compared to what commercial soap contains. According to definition, soap is a chemical compound combination produced when a metal radical interacts with a fatty acid. Any salt of those fatty acids that is water soluble and has eight or more carbon atoms is referred to be soap. The metals commonly used in soap making are sodium and potassium, which produce water laundry and cleaning products that are soluble in soap. Herbal soap preparations are medicines or pharmaceuticals because they include antibacterial and antifungal agents (Arun, 2023; Wijayawardhana *et al*., 2021).

How to formulate an herbal soap?

- **Sterilize Glassware**: Sterilize the glassware used, preferably by heat sterilization.
- **Prepare the Soap Base**: Weigh and melt 100 g of glycerin soap base in a water bath (The glycerin soap base can be prepared from coconut oil and sodium hydroxide, without alcohol).
- **Mix Plant Extracts and Other Ingredients**: In another beaker, mix the plant extract (as per the formulation design, see the next page), vitamin E oil, and other ingredients such as *aloe vera* gel, coconut oil, and honey until completely dissolved.
- **Add Fatty Acid**: Dissolve a fatty acid like stearic acid in a small amount of hot water and add it to the mixture.
- **Add Essential Oil**: To enhance the fragrance, add 1 mL of an essential oil from the desired aromatic plant.
- **Incorporate Plant Extracts**: Incorporate the plant extract mixture into the melted soap base. Stir the final mixture using a magnetic stirrer, for example.
- **Mold and Solidify**: Pour the melted mixture into separate molds and let it solidify at room temperature.

Formulation design (Arun, 2023)

Formulation Material

No.	Material	Quantity
1	Neem	10 g
2	Hibiscus powder	3.33 g
3	Aloe vera	6.6 g
4	Vitamin E	2.5 g
5	Turmeric powder	0.83 g
6	Glycerin soap base	20 g
7	Rose water	QS
8	Orange oil	1ml

1. Hibiscus
a. Family: Mallows
b. Chemical constituents: anthocyanins and polyphenols
c. Use: Hibiscus is a powerful antiaging plant with a reputation for increasing skin elasticity.
d. Color: Red

2. Neem
a. Family: Mellaceae
b. Chemical constituents: Nimbin, Nimbinene
c. Use: Anti-bacterial
d. Color: Green

3. Aloevera
a. Family: Liliaceae.
b. Chemical constituents: lignin, vitamin, enzymes, minerals.
c. Use: Anti-Aging.
d. Color: Green

4. Vitamin E: Capsule
Use: helps maintain healthy skin and eyes and strengthen the body's natural defense against illness and infection.

5. Turmeric powder
a. Family: Zingiberaceae.
b. Chemical constituents: Curmin, Dimethoxy Curmin.
c. Use: antibacterial.
d. Color: Yellow

6. Melt & Pour Soap Base
Use:
Keep skin acne free.
Prevents premature ageing.
Heals skin infection quickly.
Repair damaged skin faster.

☞ **For more details, see:** Arun, 2023; Rani *et al*.., 2023 and Wijayawardhana *et al*., 2021, etc.

B- How to prepare herbal Creams?

Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external application (Sonalkar & Nitave, 2016).

Example: Formulation and Evaluation of Multipurpose Herbal Cream (Navindgikar *et al*., 2020).

The goal of the research was to develop an herbal cream for moisturizing, nourishing, whitening, and treating various skin diseases. *Curcuma longa* (Turmeric powder), *Carica papaya* (Papaya), *Aloe barbadensis* (Aloe-vera leaves), *Azadirachta indica* (Neem leaves), and *Ocimum sanctum* (Tulsi leaves) are some of the basic drugs used to make the *Multipurpose* cream.

Formulation material (Ingredients).

S. No.	Ingredients	Formulation F1H	Formulation F2H	Formulation F3H
1.	Aloe Vera gel	1.5 ml	1 ml	1 ml
2.	Neem extract	0.5 ml	0.2 ml	0.4 ml
3.	Tulsi extract	1.5 ml	1 ml	1 ml
4.	Beeswax	3 g	3.5 g	3.2 g
5.	Liquid paraffin	10 ml	15 ml	12 ml
6.	Borax	0.2 g	0.4 g	0.3 g
7.	Methylparaben	0.02 g	0.04 g	0.03 g
8.	Distilled Water	Q. S	Q. S	Q. S
9.	Rose oil	Q. S	Q. S	Q. S

Formulation of cream

- **Heat the Oil phase:** Heat liquid paraffin and beeswax in a borosilicate glass beaker at 75 °C and maintain that heating temperature.
- **Prepare the Aqueous phase:** In another beaker, dissolve borax, methylparaben in distilled water and heat this beaker to 75 °C to dissolve borax and methylparaben and to get a clear solution.
- **Combine phases:** Slowly add the heated aqueous phase to heated oily phase (Ashara *et al*., 2013).
- **Incorporate herbal ingredients:** Add a measured amount of aloe Vera gel, Neem extract, and Tulsi extract and stir vigorously until it forms a smooth cream.
- **Add Fragrance:** Add few drops of rose oil for fragrance.

- **Final Mixing:** Transfer the cream on the slab and add few drops of distilled water if necessary and mix the cream in a geometric manner on the slab to give a smooth texture to the cream and to mix all the ingredients properly.

Conclusion

By using Aloe Vera gel, Neem and Tulsi the cream showed a multipurpose effect and all these herbal ingredients showed significant different activities. Based on results and discussion, the formulations F1H, F2H and F3H were stable at room temperature and can be safely used on the skin.

☞ **For more details, see Navindgikar *et al*., 2020.**

Problems Chapter 3

1) Why is it essential to explore the secondary metabolites of plants?
2) What are Functional Foods?
3) What are the main sources of Phytonutrients?
4) How phytonutrients can help to prevent chronic diseases?
5) Give the health benefits and role of nutraceutical in CVS diseases.
6) How edible packaging can help in the preservation of food?
7) Why edible packaging is defined as being an environmentally friendly process?
8) Can the bioactive compounds in Piper nigrum be utilized in pharmaceuticals?
9) Describe the WHO guidelines for the assessment of herbal drugs.
10) What are the primary pharmaceutical uses of benzofurans?
11) What are the possible side effects of ginseng and Ephedra?
12) Which bioactive compounds in *Aloe vera* can be used in cosmetics?
13) Give the chemical constituents and uses of Amla.
14) What are viscosity builders?

Tutorial/Practical Works 1

Methods for extraction of bioactive compounds from plant materials: an overview

Main outcomes:

By the end of this tutorial, students should be able to:

- *describe the conventional methods used to extract bioactive compounds.*
- *describe the main factors that influence the extraction methods.*
- *explain the limitations of each method.*

1. Introduction:

Since phytochemicals make up less than 10% of the plant matrix (*Harjo et al*., 2004), their use in different commercial sectors such as pharmaceutical, food and cosmetic industries signifies the need for more appropriate and standard methods to extract these active components (Smith, 2003; Sasidharan *et al*., 2011).

It is worth important to note that the phytochemical content of plants used may vary depending on the species or organ (e.g., roots, leaves, flowers, and fruits), etc., therefore extraction conditions used may also vary.

In this tutorial, we will focus only on the conventional extraction methods.

2. Theory and principles of extraction

What is extraction used for?

- Extraction is a process used to isolate a desired substance from a mixture of substances. Extraction is often used in the laboratory to separate and purify compounds from natural sources such as plants, animals, or minerals.
- The main aim of extraction is to maximize the yield of compounds of interest, while minimizing the extraction of undesirable compounds.

Which is better to use, fresh or dry plant material?

Traditionally, **fresh plant material** was used for the extraction of phytochemicals. However, nowadays this is not as common, as it requires very rapid post-harvest processing to avoid degradation of the plant. The extraction of phytochemicals from plants is now mostly done using **dried plant** as the starting material in order to **inhibit the metabolic processes**, which can cause **degradation of the active compounds**, therefore extending the shelf life of the plant material.

Which extraction technique should I use?

The most appropriate extraction technique for plant material is solid-liquid extraction. This technique involves the use of different solvents to extract compounds from the plant material. This technique is commonly used to extract bioactive components from plants.

This section will first cover conventional methods used for the extraction of phytochemicals from plants and it will then focus on the factors that influence the quality of the resulting extract.

2.1. Conventional extraction methods

Solid-liquid extraction methods used for the extraction of phytochemicals from plants include *maceration*, *infusion*, *decoction,* and *Soxhlet* extraction.

All of these extraction processes involve:

- Firstly, the diffusion of the solvent into the plant's cells,
- Then the solubilization of the phytochemicals within the plant matrix, and
- Finally, the diffusion of the phytochemical-rich solvent out of the plant cells.

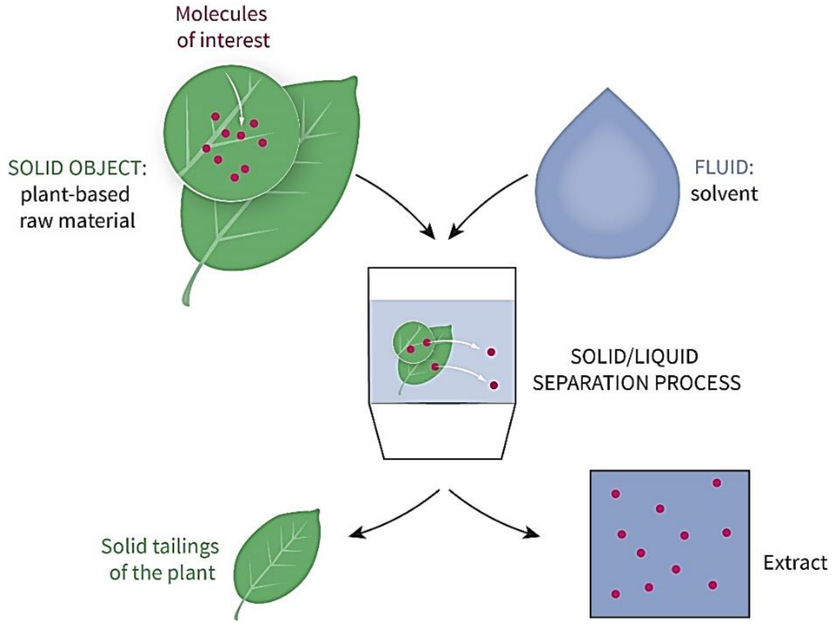


Figure 1. Phases of plant extraction processes.

This section will also cover the extraction of essential oils from plants using *steam* and *hydrodistillation*.

2.1.1. Maceration

Maceration[1] is produced by steeping the plant material in a liquid, which is generally an organic solvent, at room temperature. For this extraction process:

- The plant material is soaked in the solvent (called *Menstruum*) in a closed container.
- Prior to extraction, the plant materials can be ground to improve the contact surface/area for efficient mixing with solvent.
- The plant material and extracting solvent can be in the ratio of 1:10 w/v (Stojanovi & Veljkovi, 2007).
- The solution can be stirred (with a magnetic stirrer) to increase the rate of extraction of the phytochemicals.
- After extraction is complete, the plant material is separated from the solvent by filtration.
- The solid residue (called ***Marc***) is also pressed to recover the remaining occluded solution.

The plant material can then undergo another extraction step by adding fresh solvent to the material and letting it soak. This step can be repeated several times to ensure complete extraction of bioactive components, however it is a very time and solvent consuming process.

Maceration can take from hours to days for a single extraction, and can take weeks for repeated maceration of the plant material (Seidel, 2006). Although it is time consuming, maceration is a useful extraction method for heat-labile compounds as it is carried out at room temperature.

Filtration.

Filtration can be defined as the separation of solid from a fluid or liquid by means of a porous medium (called a filter) that retains the solid but allow the fluid to pass (which is called the filtrate).

[1] Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.).

Table 1. Merits and demerits of maceration.

Merits	Demerits
• Is quick and simple to perform, requiring minimal equipment. • It is an effective technique for extracting large amounts of compounds from plant material. • The process is relatively low-cost, as the materials needed are generally inexpensive and readily available. • It is a relatively safe technique that does not require the use of hazardous[2] chemicals.	• Is not effective for extracting compounds that have low solubility or are not readily soluble in the solvent used. • The process can be time consuming and labor intensive. • The number of compounds extracted can vary significantly depending on the solvent used and the length of time the sample is macerated. • The process can produce cloudy solutions and may not yield the desired results.

2.1.2. Infusion

Infusion is a similar process to maceration but the extraction is carried out at a set temperature (*normally higher than room temperature and up to 100 °C*) for a set period of time (from minutes to hours) and water is generally used as the extraction solvent. As for maceration, after extraction is complete the mixture is filtered.

Traditionally, infusions were made by using *boiling water* as the extracting solvent, for example, making a cup of mint tea or. Following immersion, in boiling water the plant material was left to steep and finally filtered to remove the plant material from the extract (Harbourne *et al.*, 2013).

Table 2. Merits and demerits of infusion.

Merits	Demerits
• It is a simple method that produces a uniform and consistent result. • It is easy to replicate and adapt recipes according to the desired strength and flavor. • It is relatively inexpensive and requires minimal equipment.	• The technique is not precise and can lead to inconsistent results if not done correctly. • The technique requires a lot of patience and attention to detail. • It is not appropriate for the extraction of heat-labile molecules.

N.B.- The infusion procedure usually requires a shorter time to accomplish than the maceration. This method produces plant extracts that are volatile and quickly dissolve or release their active components.

[2] Hazardous = ˈhazərdəs, risky or dangerous.

2.1.3. Decoction

Plant parts that are tougher to process, for instance barks, roots, and seeds, are typically utilized in **decoctions**.

Preparing the decoction begins with the grinding or crushing of the entire root and bark, as well as the seeds. It is important to boil the targeted portions for 30 min, or just until 50% of the water has dissipated, to produce the necessary chemicals.

To prevent the evaporation of essential components, the vessel must be covered while being heated. After the extract is removed from the heat and filtered through a filter, the decoction can be utilized whole or diluted.

This extraction method is useful for extracting thermally stable and water-soluble components from raw plant matter. The extract is concentrated before being filtered or further treated.

Table 3. Merits and demerits of decoction.

Merits	Demerits
• Process is convenient and cost-effective, • It can be used to extract a variety of compounds, including essential oils, aromatic compounds, essential fatty acids, and other active components	• It is a time-consuming method; it can take up to several hours to complete. • The procedures are straightforward, but a huge volume of solvent is required.

2.1.4. Soxhlet extraction:

In a Soxhlet[3] extraction system the sample is placed in a holder called "thimble" which is filled with the freshly condensed solvent. There is a collection flask below the thimble and a reflux condenser above it.

- Extraction starts by applying heat to the flask containing solvent; the solvent evaporates and travels to the reflux condenser above the thimble-holder.
- The condensed solvent then falls into the thimble containing the plant material.

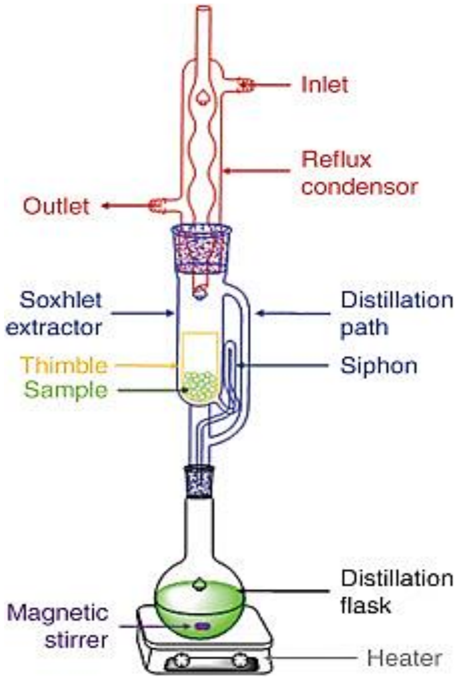


Figure 2. Assembly of a conventional Soxhlet apparatus.

[3] Soxhlet extraction was developed by Dr. Franz von Soxhlet >140 years ago for the extraction of milk fats.

- The amount of solvent keeps rising till the overflow level beyond which it is siphoned to the distillation flask.
- The solute is separated from the solvent by distillation, as the solute is left in the flask and fresh solvent passes into the plant material.

This procedure is repeated until complete extraction of plant material is achieved (Wang & Weller, 2006). Solvent and particle size will need to be selected depending on the phytochemicals, which need to be extracted. Although the Soxhlet assembly is a batch (discontinued) system, the extraction procedure is continuous in nature.

Table 4. Merits and demerits of Soxhlet extraction.

Merits	Demerits
• It is a very effective extraction method for the determination of lipids from a wide variety of biological samples. • It is simple, rapid and can be used for large numbers of samples. • It is suitable for analyzing samples that contain large amounts of fat. • The technique is also effective for the extraction of other components such as proteins, carbohydrates and vitamins.	• It is labor intensive and time consuming. • It requires a significant amount of instrumentation and expertise. • It can be expensive due to the cost of the necessary equipment and reagents. • It is not suitable for the extraction of small molecules such as fatty acids. • Difficulty in automation

2.1.5. Steam and hydrodistillation

Steam and **hydrodistillation** are extraction techniques used to extract water-insoluble volatile constituents from various matrices, including the extraction of essential oils from plants and are widely used in the perfume industry for extraction of essential oils.

For steam distillation, the steam is percolated through the plant material. The steam dissolves the essential oil in the plant material and then enters a condenser. The mixture of condensed water and oil is collected and finally separated by decanting.

For hydrodistillation, the only difference is that the plant material is submerged in the water, which is then heated until it boils. The extraction conditions can be optimized by modifying the distillation time and temperature.

The conditions may also need to be modified depending on the material being extracted, for example, for the extraction of tough material (roots or bark) glycerol may be added to the water to assist extraction (Seidel, 2006). However, for many medicinal plants the conditions used to extract essential oil are well defined in the European Pharmacopoeia (2004) in https://www.edqm.eu/en/background-and-mission.

3. Factors affecting extraction methods

The efficiency of the solid-liquid extraction methods is affected by factors such as solvent type, the ratio of solvent to plant material, temperature, time, and matrix structure (e.g., particle size, plant organ).

3.1. Solvent

The extraction of phytochemicals is dependent on the dissolution of each compound in the plant material matrix and their diffusion into the external solvent (Shi *et al.*, 2005), therefore, the choice of extraction solvent is one of the most important matters to consider for solid-liquid extraction. The factors that need to be considered when choosing the solvent or solvent system for the extraction of phytochemicals are the safety of the solvent and potential for the formation or extraction of undesirable compounds and finally solubility of the target compounds (Seidel, 2006).

N.B. In recent years, organic solvents (e.g., methanol) have been used to extract phytochemicals from plant material (Naczk & Shahidi, 2004). These extraction procedures were efficient and resulted in high yields of phytochemicals, but the solvents may be harmful to human health if ingested and therefore would not be desirable for inclusion in food or beverage.

To produce phytochemical-rich extracts for incorporation into foods and beverages it is necessary to use food-grade solvents (e.g., water, ethanol, or mixtures of these).

A variety of solvents is employed to separate the desired components from various natural sources (**Table 5**).

Table 5. Types of solvents Used for Bioactive Component Extraction

Type of solvent	Bioactive compound
Acetone	Flavonols
Chloroform	Terpenoids, Flavonoids
Dichloromethanol	Terpenoids
Ethanol	Tannins, Polyphenols, Flavonol, Terpenoids, Alkaloids
Ether	Alkaloids, Terpenoids
Methanol	Anthocyanins, Terpenoids, Saponins, Tannins, Flavones, Polyphenols
Water	Anthocyanins, Tannins, Saponins, Terpenoids

Solvents such as ***ethanol***, ***methanol***, or ***ethyl-acetate*** are preferred for extraction of hydrophilic compounds; whereas, ***dichloromethane*** or a mixture of methanol and dichloromethane is preferred for the extraction of hydrophobic compounds. In a few cases, extraction with ***hexane*** is used to remove chlorophyll (Cos *et al.*, 2006**)**.

3.2. Temperature

Many studies have investigated the effect of temperature on the extraction of polyphenolics from plant material (Labbe *et al*., 2006; Lim & Murtijaya, 2007). In general, a higher extraction temperature causes an increase in the rate of diffusion of the soluble plant phytochemicals into the extraction solvent, thereby reducing extraction time.

An increase in temperature can cause an increase in the concentration of some phytochemicals, which is possibly due to an increase in the solubility of many of these bioactive compounds, or to the breakdown of cellular constituents result in the release of the phytochemicals (Lim & Murtijaya, 2007). In addition, an increase in the extraction temperature may also inhibit enzymatic activities thus resulting in an increase in the yield of the bioactive compounds.

The temperature used for extraction will be limited depending on the extraction solvent chosen as they all have different boiling points, for example, the boiling point of acetone is 56–57 °C whereas the boiling point of water is 100 °C.

3.3. Time

The time it takes for extraction of phytochemicals will vary depending on the plant species to be extracted, the particle size of the material and the plant organ. For example, the extraction of phytochemicals from leafy material will be faster than the extraction from harder material such as roots or bark (Whitehead, 2005).

3.4. Particle size

Plant material can undergo grinding or milling before extraction to reduce the particle size. The smaller the particle size of the material the shorter the path that the solvent has to travel, which decreases the time for maximum phytochemical content to be extracted (Shi *et al*., 2005).

The disadvantage of grinding or milling the plant before extraction is that plant material of small particle size may block filters quicker than bigger particles and this could possibly result in wastage of the extract and extended extraction times (Whitehead, 2005).

4. Limitations of conventional extraction techniques

In general, the disadvantages of using conventional extraction techniques that are commonly cited in the literature include long extraction times, requirement of large quantities of solvent and the degradation of heat labile phytochemicals by using high temperatures for extraction.

N.B. As an alternative to conventional methods, advanced extraction techniques (such as enzyme assisted, microwave assisted, ultrasound assisted and supercritical extraction techniques) have resulted in increased yield within a shorter duration. Nevertheless, the scale-up of the advanced techniques remains a challenge for further research and development.

5. Conclusion

By varying the factors affecting extraction, including solvent, temperature, particle size, solvent to solid ratio and time, a wide range of phytochemicals can be extracted using conventional methods. However, the extraction parameters must be optimized depending on the bioactives of interest, the plant species and the plant organ, as the extraction conditions can vary greatly.

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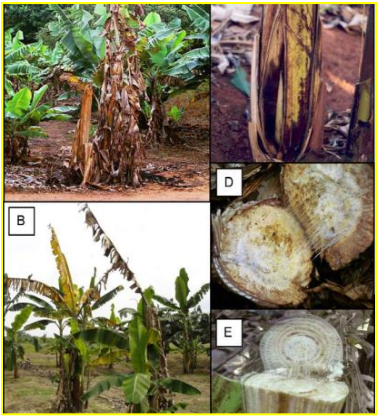
Tutorial /Practical Works 2 – (Paper analysis)

Extraction and quantification of phenolic compounds in oil palm roots

Learning outcomes:
By the end of this tutorial, students should be able to:

- Read and understand research papers.
- Quantify the amount of PSMs (i.e., polyphenols) by measuring light absorption.
- Analyze and discuss the results of a scientific experiment

1. Prelude:



Fusarium wilt is the most serious disease of oil palm (*Elaeis guineensis* Jacq). A soil fungus called *Fusarium oxyporum* causes it.

In order to examine the impact of Fusarium wilt on the production of polyphenols in oil palm, a comparative study of extraction, quantification of phenolic compounds was carried out on the roots of this economic species (Diabaté *et al*., 2009).

2. Methods of extraction of phenolic compounds:

Two methods of phenol extraction from roots with or without inoculation with *Fusarium* oxyporum were used:

2.1. Conventional extraction method:

In this method, 5 g of fresh roots were cut and ground in 40 mL of pure Ethanol using a Mixer. The maceration of the tissues in pure Ethanol was carried out at room temperature for 24 hours. The ground root-Ethanol mixture is then filtered. The filtrate is dried using a rotary evaporator at 40° C, and taken up in 4 mL of pure Ethanol. The extract thus obtained is stored in the refrigerator at 4°C (to prevent the action of polyphenoloxidases) before the assay.

2.2. Modern extraction method:

In this new extraction method, 5 g of roots are ground in liquid nitrogen (-160°C) and taken up in 40 mL of an Ethanol-Water mixture (70-30; v/v) in the presence of 1 mL of an antioxidant, Sodium MetaBisulphite (SMB) at 2% in water. This mixture is boiled for 30 minutes. After cooling, the liquid obtained is filtered and dried using a rotary evaporator at 40°C. The residue is taken up in 4 mL of the Ethanol-Water mixture (70-30) + 0.1mL of 2% SMB. The extract obtained is stored in the refrigerator at 4°C before the assay.

3. Quantification of phenolic compounds:

The content of phenols in the two root extracts is measured by the Folin-Ciocalteu method (Singleton & Rossi, 1965). This method measures the redox potential of phenols. For that, 0.1 mL of each of the two extracts is mixed with 2 mL of a freshly prepared 2% sodium carbonate (Na_2CO_3) solution; the whole is stirred by a vortex. After 5 min, 100 µL of 1N Folin-Ciocalteu reagent are added to the mixture, the whole is left for 30 min at room temperature and away from light. The reading is carried out against a blank using a spectrophotometer at 700 nm. A calibration curve is produced in parallel under the same operating conditions using **chlorogenic acid** as a positive control (Vermerris & Nicholson, 2006).

The results are expressed in mg equivalent of chlorogenic acid as a reference and are expressed in mg of equivalent chlorogenic acid per gram of fresh weight (mg CGA/g FW).

4. Results and interpretation

Inoculation time	Extract		CGA (mg/L)	O.D (Absorbance)
	Ext 1	Ext 2		
D0	0.28	0.98	1	0.44
D1	0.25	0.95	2	0.48
D3	0.35	0.98	3	0.80
D5	0.46	1.02	4	1.12
D8	0.38	0.98	5	1.45

- Plot the calibration curve of absorbance versus concentration of CGA. (Watch this video to learn how to create a linear standard curve 💻 https://www.youtube.com/watch?v=n-yrO1A9zbs)
- Calculate the polyphenol concentration for the two extracts at different inoculation times.
- Represent the results as a histogram.
- Interpret your results regarding the method of extraction and the time of inoculation.

5. References:

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Good luck ☺

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APPENDIXES

HOMEWORK N°1

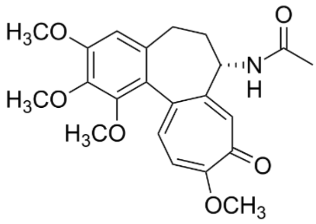
☞ **Prepare a data-sheet as in the example below for each of the following products:**

Morphine; Vinblastine; Atropine; Caffeine; Nicotine; Cocaine; Piperine; Rosmarinic acid; Resveratrol; Lignans.

Colchicine

Nature and main source:
Colchicine, is the main alkaloid of *Colchicum autumnale* (*Liliaceae*).

Chemical structure:



Use in medicine or biological activities:
Colchicine is effective in gout and familial Mediterranean fever. Colchicine has been used for a wide spectrum of dermatological disorders, including chronic urticaria, cutaneous vasculitis, actinic keratosis, acne vulgaris, palmoplantar pustulosis, psoriasis, and aphthous stomatitis [1].

Mechanism of action if any:
Although the exact mechanism of its action is not fully understood, colchicine accumulates in leucocytes and modulates the production of chemokines and prostanoids, decreasing neutrophil degranulation, chemotaxis, and phagocytosis [2].

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Good Luck☺

HOMEWORK N°2

☞ **Answer the following questions:**

1. When are the plant bioactive molecules first isolated and used for diseases treatment?
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2. Who first used the term aromatherapy?
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3. What do you get after steam distillation of an herbal drug?
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4. Why do we need to control the harvesting and drying of aromatic and medicinal plants? What are the conditions that allow good conservation of the plant bioactive molecules?
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5. Why should we avoid picking wild plants in places where there may be fertilizers or pesticides, or on the side of paths or roads?
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6. Why do we need to know plants well before picking them?

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7. Why should compost be used as a fertilizer, to grow aromatic and medicinal plants in your garden, for example?
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8. Why do we proceed by infusion or decoction for a given plant? Which parts of the plant will undergo a decoction or infusion?

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9. What is the difference between vegetable oil and essential oil? Why is a plant macerated in vegetable oil?

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10. Why should essential oils be used with caution? And why should an essential oil be mixed with a vegetable oil (e.g. almond oil) before applying it to the skin?

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11. Why does a dry plant keep better and keep its active ingredients intact? Why do leaves and flowers keep less well?

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Good Luck☺