Consortium for Educational Communication

Module
on
Secondary Metabolites

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TEXT

Introduction

The chemical reactions that take place in any organism is called metabolism. The food that the organisms contains carbon, nitrogen, and mineral gets converted into energy. The energy produced ends up in molecules that are common to all cells .These molecules are required for the proper functioning of cells and organisms., e.g., lipids, proteins, nucleic acids etc. and carbohydrates. They are also called **primary metabolites** or biomolecules. The primary metabolites are involved in photosynthesis, respiration, protein and lipid metabolism. Most plants also spend carbon and energy for the synthesis of molecules that do not seems to have any role in normal cell function. These molecules are known as **secondary metabolites**.

Role of Secondary Metabolites:

- *They Induce plant defence against insect infestation*
- *They help to recognize specific components of insect saliva*
- *Some of these compounds inhibit herbivore digestion*
- *Herbivore-induced volatiles have complex ecological functions*
- *They behave as antimicrobial compounds against pathogen attack*
- *Infection induces additional antipathogen defence*
- *Phytoalexins increase after pathogen attack*
- *Some plants recognize specific pathogen-derived substances*
- *A single encounter with a pathogen may increase resistance to future attacks.*

Types of plant derived secondary metabolites.

Plant secondary metabolites can be divided into three chemically distinct groups:

- Terpenes
- Phenolics
- Nitrogen-containing compounds. This part is dealt as a separate chapter hence not detailed hereunderPaper 2:Food biochemistry titled - Alkaloids

Terpenes

The terpenes, or terpenoids, are the largest group of secondary metabolites produced by plants. Most of the compound in this class are insoluble in water. Terpenes may or may not have well defined functions in the plants. Eg. Gibberellins, a diterpenes is an important plant hormone, these are primary metabolites. The vast majority of terpenes, however, are secondary metabolites presumed to be involved in plant defence.

Terpene deters the feeding of herbivorous and are toxins tomany herbivorous insects and mammals.Thus, they play as defence molecules in the plant kingdom. Eg. *Pyrethroids* (Monoterpene esters) found in the leaves and flowers of *Chrysanthemum* species, have insecticidal activity. Monoterpenes accumulation in resin ducts ofconifers such as pine and fir are abundant in the needles, twigs, and trunk. These compounds are toxic to bark beetles which are serious pests of conifer species throughout the world and tonumerous other insects.Essential oil is a mixtures of volatile compounds commonly in plants, they lend a characteristic odour to their foliage. Essential oils have well-known insect repellent properties e.gPeppermint, lemon, basil (tulasi), and lemon grass.Monoterpene of lemon oil iscalled limonene and that of peppermint oil is menthol. The presence of both lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent like properties.

Both natural and synthetic pyrethroids are popular ingredients in commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals.

Phenolic compounds

Most of the plants produce a large variety of secondary compounds that contain a phenol group. These substances are classified as *phenolic compounds, or phenolics*. Plant phenolics are a chemically heterogeneous group of nearly 10,000 individual compounds: Some are soluble only in organic solvents, some are water-soluble carboxylic acids and glycosides, and others are large, insoluble polymers. Phenolics play variety of roles in the plant due to their chemical diversity. Many serve as defense against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or in reducing the growth of nearby competing plants. One of the major group of phenolic compounds are Tannins. Tannins are astringents, bitter plant polyphenols that either bind and precipitate or shrink proteins.These tannins are usually dividedin to hydrolysable tannins and condensed tannins (Proanthocyanidins).Tannins, the most abundant and widely distributed phenolic polymers of higher plant, combine with proteins, polysaccharides, etc. to from complexes, thereby increasing their resistance to microbial degradation.

There are two major groups – hydrolysable and condensed tannins (**Fig 1 and 2, Table 1).** Hydrolysable tannins are composed of esters of gallic acid (gallo-tannins) or ellagic acid (ellagitannins) with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric products.

The major commercial hydrolysable tannins are extracted from Chinese gall (*Rhus semialata*), sumac (*Rhus coriara*), Turkish gall (*Quercus infectoria*), tara (*Caesalpina spinosa*), myrobalan nuts (*Terminalia chebula*), and chestnut (*Castanea sativa*). Condensed tannins, also known as polymeric proanthocyanidins, are composed of flavonoid units, and are usually more abundant in tree barks and woods than their hydrolysable counterparts. The important commercial condensed tannins are extracted from wattle (*Acacia mollissima* and *A. mearnsii*), quebracho (*Schinopsis lorentzii* and *S. balansae*) and tree barks. Some of the therapeutic importance of plant phenolic are listed in **table 2.**

The colored pigments of plants help to attract pollinators and seed dispersers. The coloured pigments are of two types: *carotenoids and flavonoids*. Carotenoids are yellow, orange, and red terpenoids compounds.Carotenoids also serve as an accessory pigments in photosynthesis. The flavonoids include a wide range of colored compounds. The most widespread group of pigmented flavonoids is the *anthocyanins. Anthocyanins are* responsible for most of the red, pink, purple, and blue colorseen in flowers and fruits. The other two groups of flavonoids like *flavones and flavonols* are commonly found in flowers. These flavonoids absorb light at shorter wavelengths than anthocyanins, hence, they are not visible to the human eye. However, insects such as bees, which can see the ultraviolet range of the spectrum respond to flavones and flavonols.*Isoflavonoids*, which are found mostly in legumes, have several different biological activities. Some, such as rotenone, can be used effectively as insecticides, pesticides (e.g., as rat poison), and piscicides (fish poisons). Other isoflavones have anti-estrogenic effects; for example, sheep grazing on clover rich in Isoflavonoids often suffer from infertility. Isoflavones may also be responsible for the anticancer benefits of foods prepared from soybeans.

A second category of plant phenolic polymers with defensive properties, besides lignin, is the *tannins*. They are general toxins that can reduce the growth and survival of many herbivores when added to their diets. In addition, tannins act as feeding repellents to a great variety of animals. Mammals such as cattle, deer, and apes characteristically avoid plants or parts of plants with high tannin contents. Unripe fruits, for instance, frequently have very high tannin levels, which deter feeding on the fruits until their seeds are mature enough for dispersal. Herbivores that habitually feed on tannin-rich plant material appear to possess some interesting adaptations to remove tannins from their digestive systems. Plant tannins also serve as defence against microorganisms.

From leaves, roots, and decaying litter, plants release a variety of primary and secondary metabolites into the environment. The release of secondary compounds by one plant which has effect on neighbouring plant is referred to as allelopathy.If a plant can reduce the growth of nearby plants by releasing chemicals into the soil, it may increase its access to light, water, and nutrients. Allelopathy is currently of great interest because of its potential agricultural applications. Reductions in crop yields caused by weeds or residues from the previous crop may in some cases be a result of allelopathy. An exciting future prospect is the development of crop plants genetically engineered to be allelopathic to weeds.

Beneficial effects of plant phenolic's on humans

- Anti-coagulant activity, akin to aspirin
- It has a preventative action against cancer, combating the development of tumours
- It is a powerful anti-oxidant (20 times more than vitamin-E)
- It has anti-inflammatory effects.
- It facilitates the reaction of the immune system.

However all these effects are under the administration of small quantities of these phenolic compounds. Higher quantities are poisonous.

Bioavailability of polyphenols

It is important to realize that the polyphenols that are themost common in the human diet are not necessarily the most activewithin the body, either because they have a lower intrinsicactivity or because they are poorly absorbed from the intestine,highly metabolized, or rapidly eliminated. In addition, themetabolites that are found in blood and target organs and thatresult from digestive or hepatic activity may differ from thenative substances in terms of biological activity. Extensiveknowledge of the bioavailability of polyphenols is thus essentialif their health effects are to be understood.

Metabolism of polyphenols occurs via a common pathway.The aglycones can be absorbed from the small intestine. However,most polyphenols are present in food in the form of esters,glycosides, or polymers that cannot be absorbed in their nativeform. These substances must be hydrolyzed by intestinal enzymesor by the colonic microflora before they can be absorbed. Whenthe flora is involved, the efficiency of absorption oftenreduced because the flora also degrades the aglycones, which itreleases to produce various simple aromatic acids in the process.During the course of absorption, polyphenols are conjugatedin the small intestine and later in the liver. This processmainly includes methylation, sulfation, and glucuronidation. This is a metabolic detoxication process common to many xenobioticsthat restricts their potential toxic effects and facilitatestheir biliary and urinary elimination by increasing their hydrophilicity.The conjugation mechanisms are highly efficient, and aglyconesare either absent in blood or present in low concentrationsafter consumption of nutritional doses. Circulating polyphenolsare conjugated derivatives that are extensively bound to albumin.Polyphenols are able to penetrate tissues, particularly thosein which they are metabolized, but their ability to accumulatewithin specific target tissues needs to be further investigated.Polyphenols and their derivatives are eliminated chiefly inurine and bile. Polyphenols are secreted via the biliary routeinto the duodenum, where they are subjected to the action ofbacterial enzymes, especially ß-glucuronidase,inthe distal segments of the intestine, after which they may bereabsorbed. This enterohepatic recycling may lead to a longerpresence of polyphenols within the body.

*Nitrogen-containing compounds:*These compounds are commonly known as alkaloids. (This part is dealt as a separate chapter hence not detailed herePaper 2: FOOD BIOCHEMISTRY (1st year) - Alkaloids)

Secondary metabolites from microbes.

In the microbial system the phase of growth is well defined as shown in **Fig3**. Primary metabolites are involved in growth, development, and reproduction of the organism. The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite. Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are considered essential for proper growth. Examples of primary metabolites include alcohols such as ethanol, lactic acid, and certain amino acids.

Secondary metabolites are typically organic compounds produced through the modification of primary metabolite synthases. Secondary metabolites do not play a role in growth, development, and reproduction like primary metabolites. They are typically formed during the end or near the stationary phase of growth. Many of the identified secondary metabolites have a role in ecological function, including defence mechanism(s), by serving as antibiotics and by producing pigments. Examples of secondary metabolites with importance in industrial microbiology include atropine and antibiotics such as erythromycin and bacitracin.

Secondary metabolites in the microbial world serve:

(i) As competitive weapons used against other bacteria, fungi, amoebae, plants, insects, and large animals;

(ii) As metal transporting agents;

(iii) As agents of symbiosis between microbes and plants, nematodes, insects, and higher animals;

(iv) As sexual hormones;

(v) As differentiation effectors.

Secondary metabolites are not produced until the microbe has largely completed its logarithmic growth phase and entered the stationary phase of the growth cycle (**Fig 3).** Period of production is called stationary phase also known as idiophase and metabolites as idiolites.

In the idiophase, cells do not divide but are metabolically active. Idiolites are organic compounds produced only after considerable number of cells and primary metabolite have accumulated (end or near the stationary phase of growth).These are produced under sub-optimal concentrations of O_2, deviations of pH or when primary nutrient source is depleted. Though, idiolites are a characteristic feature of fungal, yeast, actinomycetes and bacterial growth but are not produced by a few strains of E.coli.In some strains secondary metabolite are produced by further conversion of a primary metabolite. Alternatively, these may be metabolic products of the original growth medium. Not necessary for growth, development, and reproduction like primary metabolites. Their production is influenced by environmental factors.

Secondary metabolites are synthesized for a definite period by cells that are no longer undergoing balanced growth. A single microbial type can produce very different metabolites.

Their production is regulated by complex biochemical pathways and some strains can produce a variety of idiolites. For example a strain of Streptomyces can produce a variety of 35 anthracyclines.

Examples of secondary metabolite from the microbial world are antibiotics, toxins and pigments to name a few.

Bacitracin, a peptide antibiotic, derived from *Bacillus subtilis*, is commonly used as a topical drug. It has a wide spectrum antibiotic against Gram positive and Gram negative bacteria. The number of antibiotic compounds from various microbial sources are summarised in **table 3.**

Common toxins from the bacterial world are exotoxins. Some examples are

1. *Corynebacterium diphtheriae*. Diphtheria.Exotoxin inhibits protein synthesis and causes cell death.

2. *Clostridium tetani*. Exotoxin blocks action of neurones of spinal cord. Causes tetanus.

3. *Clostridium perfringens*. (a) Causes gas gangrene. Exotoxin (alpha toxin) which causes cell death, (b) Enterotoxin causes hyper secretion of water and electrolytes in diarrhoea.

4. *Clostridium botulinum*. Exotoxin causes paralysis of respiratory muscles by attacking the acetylcholine of synapses and neuromuscular junctions leading to death. Causes botulism.

5. *Vibrio cholerae* 01 and 0139. Enterotoxin (Exotoxin) causes hyper secretion of water and electrolytes within gut in diarrhoea.

6. Enterotoxigenic *E. coli*. Produce enterotoxin (LT- heat labile exotoxin) causes hypersecretion of water and electrolytes within gut.

7. *Shigella dysenteriae* type 1 (Shiga bacillus). Exotoxin causes acute inflammation.

8. *Staphylococcus aureus*- some strains : (a) Toxic shock syndrome toxin-1. Causes toxic shock syndrome. (b) Staphylococcal enterotoxin causes toxin type food poisoning and stimulates vomiting centre of brain.

9. *Streptococcus pyogenes*. Pyrogenic (Erythrogenic) exotoxin causes scarlet fever and toxic shock syndrome.

Pigments from microbes are being used as a replacement for artificial colours in food, pharma and health care industries. Some of the pigments are listed in **table 4.**

Overproduction of secondary metabolites can be achieved by manipulating larger number of genes (gene cassettes).

Conclusion.

Nature produces an amazing variety and number of products. About 100,000 secondary metabolites with molecular weight less than 2500 have been characterized, mainly produced by microbes and plants, some 50,000 are from microorganisms.New bioactive products from microbes continue to be discovered at an amazing pace: 200±300 per year. Many companies have participated in the successful discovery and application of microbial secondary metabolites.

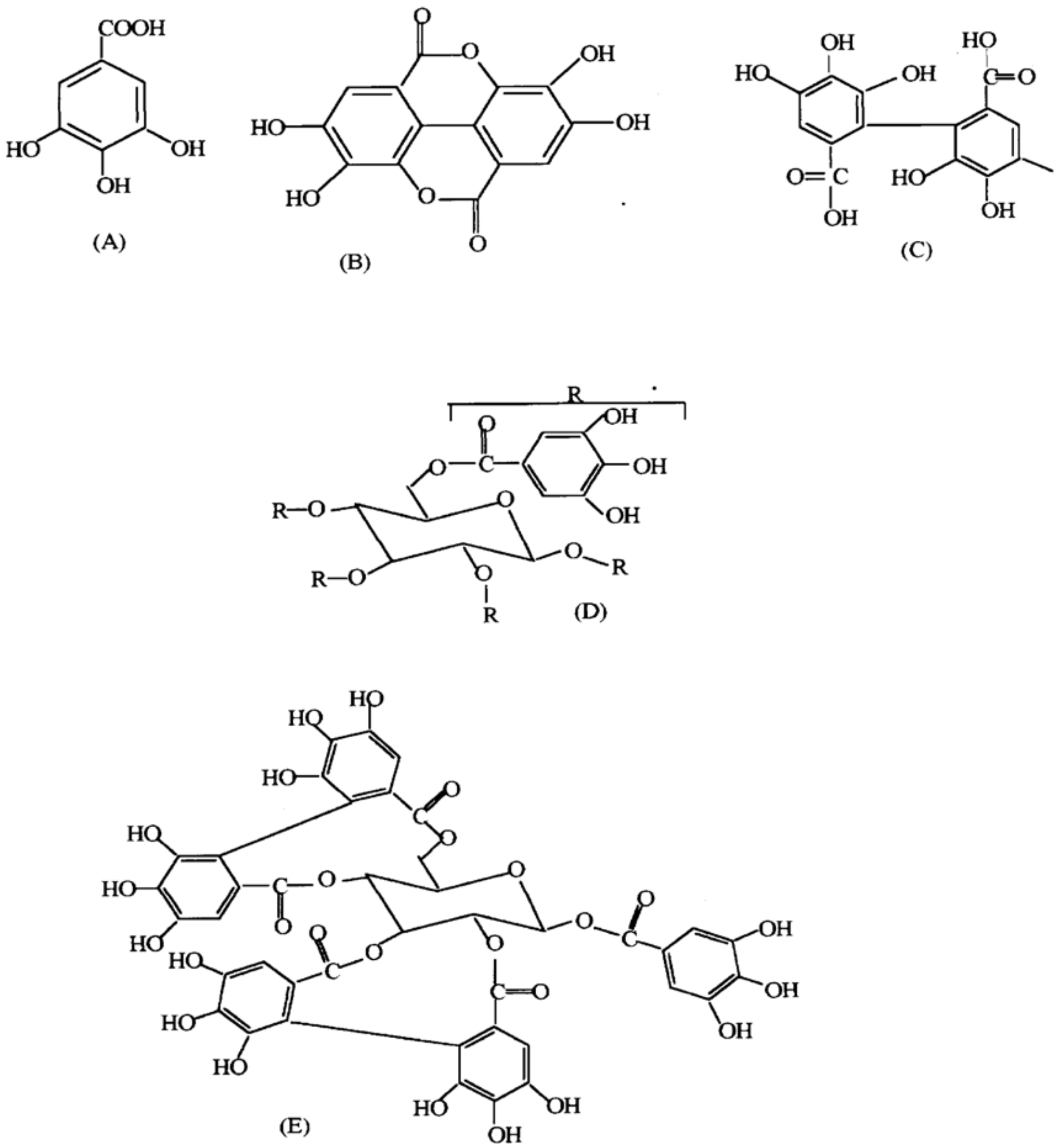

Figure 1. Structure of (A) gallic acid, (B) ellagic acid, (C) hexahydroxydiphenic acid, (D) gallotannin and (E) ellagitannin.

Fig 1:**Structure of hydrolysable tannins like (A) gallic acid, (B) ellagic acid, (C) hexahydroxydiphenic acid, (D) gallotannin and (E) ellagitannin.**

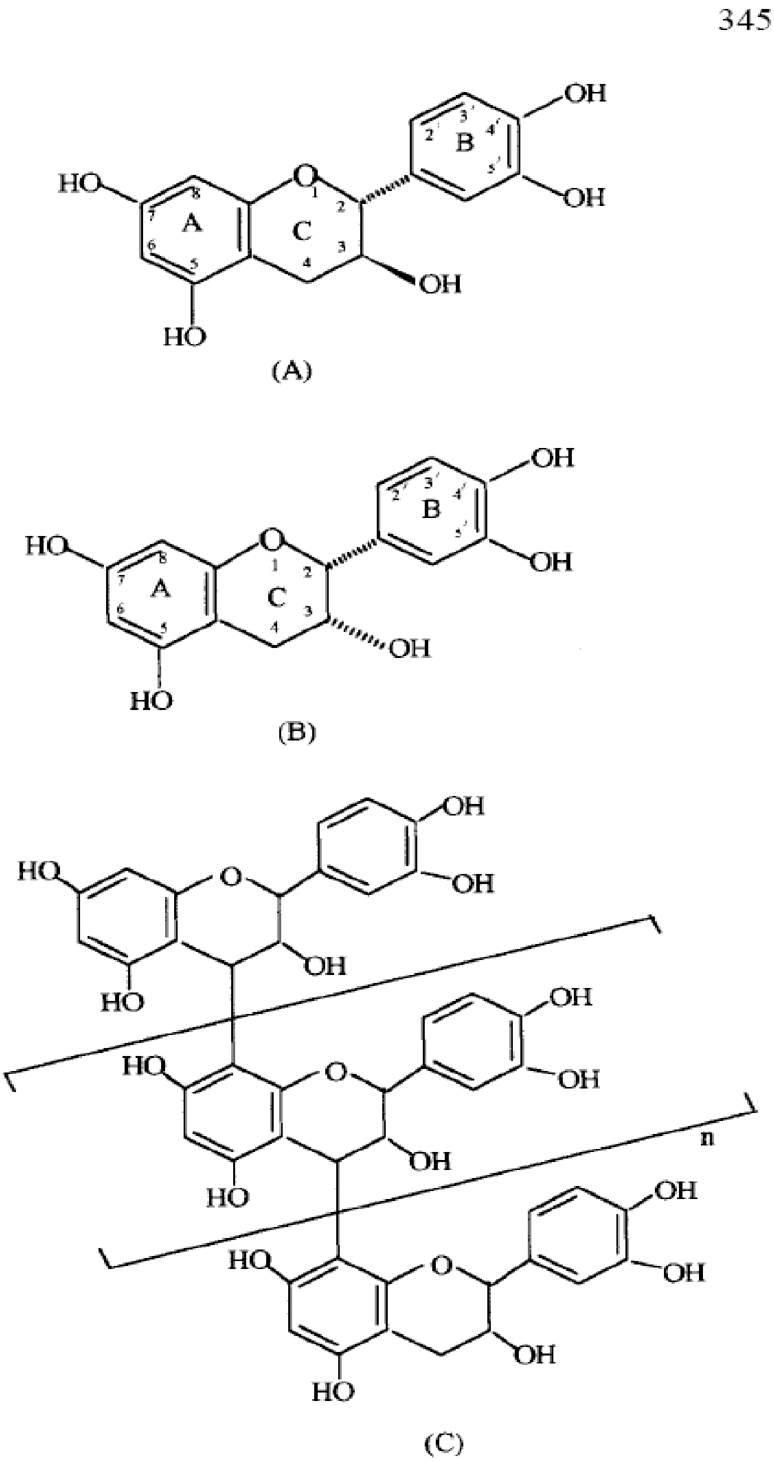

Fig 2: Structure of condensed tannin like (A) catechin, (B) epicatechin, and (c) 4,8linked procyanadin.

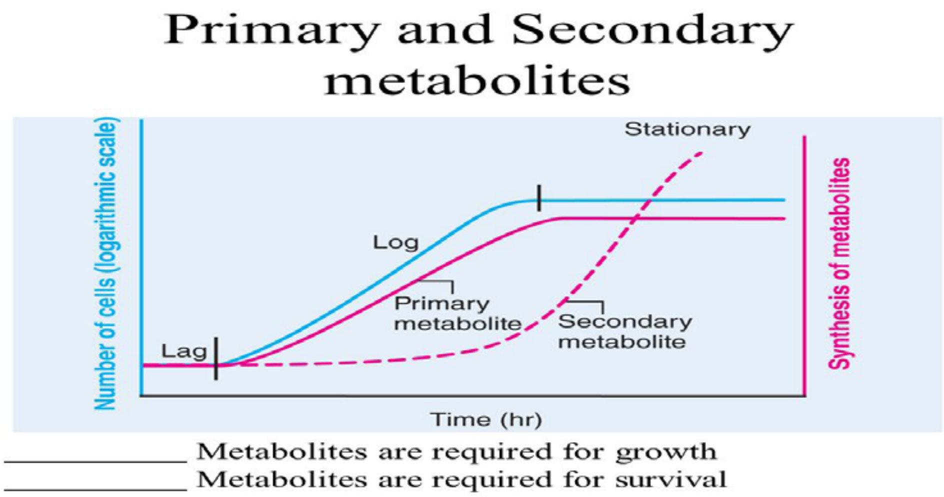

Fig 3 Growth curve along with production of primary and secondary metabolites in microbes.

Hydoysable Tannis	Catechin Tannins	Condensed Tannins
1.Gallotannins, e.g.Tannic acid(commercial name of Chinese gall tannins);Yeild gallic acid and glucose on hydrolysis	Catechin and apicatechin gallates ;yield catachin/ epicatechins and gallic acid on hydrolysis;Have properties of hydrolysable and condensed tannins	Polymeric proanthocyanidine;yield monmeric flavonoids such as flavan-3,4-diols and flovan-3-ols on hydrolysis.e.g.quebracho tannins from the wood of quebracho tree.
SOURCES: Tara pods((*Caesalpina spinosa*), gall nuts (pathological excrescences) *Quercus infectoria* (Turkish gall) &*Rhus semialata* (Chinese gall), (*Rhus coriara*)	**Sources:** Tropical shrub legumes, tea leaves.	**Sources:** Commonly found in fruits and seeds such as grapes, apple, olives, beans, sorghum grains, carob pods, cocoa & coffee, besides tree bark & heart wood.

2. Ellagitannins – yield ellagic acid & glucose on hydrolysis. **Sources:** Wood of oak (*Quercus* spp.), chestnut (*Castanea* spp.) and myrobalan (*Terminalia chebula*)		**Common types are** 1. Quebracho tannins from wood of *Schinopsis* spp., *Loxopterygium* spp. 2. Wattle tannins from *Acacia* spp. 3. Bark tannins from pine (*Pinus* spp.), (*Terminalia chebula*) oak (*Quercus* spp.) and gaboon wood (*Aucoumea kleneana*)
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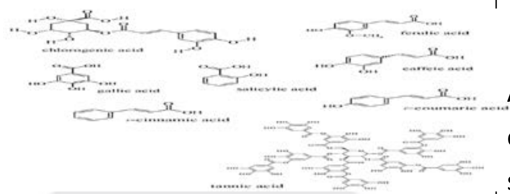
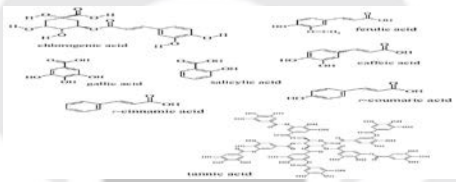
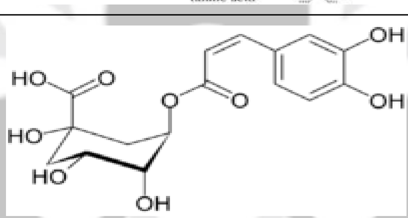
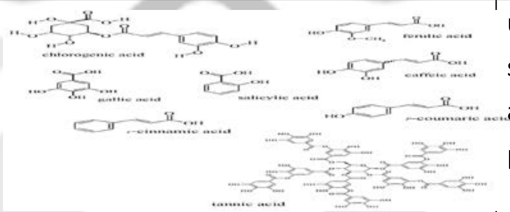
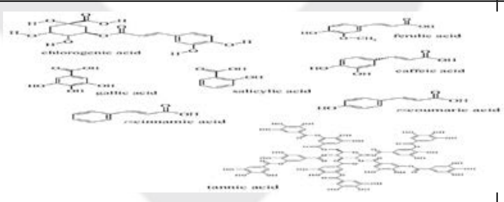
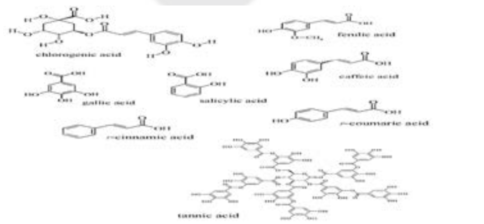
Table 2: phenolic acids from plants with their therapeutic values.

NAME	MOLECULAR FORMULA AND MOLECULAR WEIGHT	STRUCTURE	SIGNIFICANCE
Tannic acid	$C_{76}H_{52}O_{46}$ 1701.20		Anti-bacterial, Anti-enzymatic and Anti-stringent
p-Coumaric acid	$C_9H_8O_3$ 164.16		Antioxidant properties and is believed to reduce the risk of stomach cancer
Chlorogenic acid	$C_{16}H_{18}O_9$ 354.31	 **chlorogenic acid**	Antioxidant, also slows the release of glucose into the bloodstream after a meal
t-Cinnamic acid	$C_9H_8O_2$/ $C_6H_5CHCHCOOH$ 155.20		used in flavours, synthetic indigo, and certain pharmaceuticals
Caffeic acid	$C_9H_8O_4$ 180.16		act as a carcinigenic inhibitor, an antioxidant in vitro
Gallic acid	$C_7H_6O_5$ 170.12		Anti-bacterial, Anti-viral properties

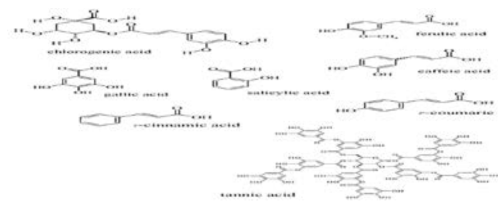
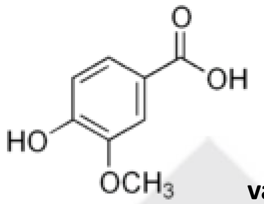
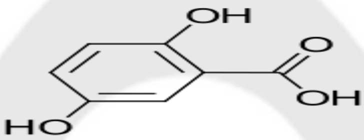
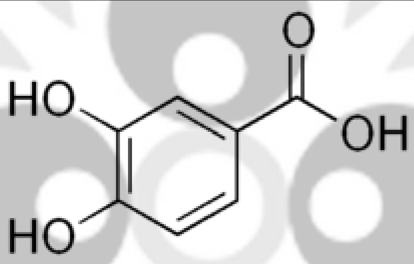
Ferulic acid	$HOC_6H_3(OCH_3)CH{=}CHCO_2H$ 194.18		precursor in the manufacture of other aromatic compounds.
Vanillic acid	$C_8H_8O_4$	 **vanillic acid**	used in Traditional Chinese medicine
Gentistic acid	$C_7H_6O_4$	 **gentistic acid**	An antioxidant recipient in some pharmaceutical preparations.
Protocatechuic acid	$C_7H_6O_4$	 **Protocatechuic acid**	prevents carcinogenesis or ant tumor growth in vivo

Table 3. Microbial secondary metabolites used as antibiotics

Type of organism	**Approximate No of compounds identified as antibiotics**
Filamentous bacteria (actinomycetes) of the genus Streptomyces	6,600
Other antinomycetes	1320
Non-Filamentous bacteria	1440
Bacterial	2640
Total	12000

Table 4: Pigments produced by different microorganisms.

Pigment	Microorganism
Indigoidine (blue-green)	Streptomyces aureofaciens CCM 323, Corynebacterium insidium
Carotenoid	Gemmatimonas aurantiaca T-277
Melanin (black-brown)	Kluyveromyces marxianus, Streptomyces chibanensis, Cryptococcus neoformans, Aspergillus sp., Wangiella dermatitidis, Sporothrix schenckii, andBurkholderia cepacia
Prodigiosin (red)	Serratia marcescens, Rugamonas rubra, Streptoverticillium rsubrireticuli, Serratia rubidaea, Vibrio psychroerythrus, Alteromonas rubra, and Vibrio gaogenes
Zeaxanthin	Staphylococcus aureus, Vibrio psychroerythrus, Streptomyces sp.,and Hahella chejuensis
Canthaxanthin (orange)	Monascus roseus, Bradyrhizobium sp.
Xanthomonadin (yellow)	Xanthomonas oryzae
Astaxanthin (red)	Phaffia rhodozyma, Haematococcus pulvialis
Violacein (purple)	Janithobacterium lividum
Anthraquinone (red)	Pacilmyces farinosus

Halorhodopsin and rhodopsin	Halobacterium halobium
Rosy pink	Lamprocystis roseopersicina
Violet/purple	Thiocystis violacea, Thiodictyon elegans
Rosy peach	Thiocapsa roseopersicina
Orange brown	Allochromatium vinosum
Pink/Purple violet	Allochromatium warmingii