Sumera Javad · Ayesha Butt *Editors*

Nanobotany

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Editors
Sumera Javad
Department of Botany
Lahore College for Women University
Lahore, Pakistan

Ayesha Butt
Department of TESOL
Lahore College for Women University
Lahore, Pakistan

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Dedicated to our parents
whose efforts and sacrifices have enabled us
to do something in life

Preface

This book is a link between botany and nanotechnology. This book is intended to fill the gap in interrelated fields of applied research for botany students.

Since last 100 years, there is an increased interest of research groups and enhanced tendency of investments in the fields of nanotechnology. Nanotechnology has really revolutionized the era and has greatly speeded up the progress of technology. Plant sciences or botany researchers have found their interest in the field of nanotechnology and once again, this renewable green treasure of nature has proved its vitality for the bulk production of nanoparticles. So, this book is an effort to link both the fields. No doubt, nanotechnology is a very broad term and field, but this study is an effort to understand the nature of nanoparticles and then practically find their applications in botany.

In this era of fast growing technology, there is need for bulk production of nanoparticles. So, there is a concept of this production with the help of plant metabolites. There are details of mechanisms which can be opted to develop nanoparticles with controlled size and shape with plant metabolites. It is a special effort by different scientists from various fields of life.

It is expected that this book will be equally resourceful for the botany students as well as for the nanotechnologists. This book will have an academic impact for graduate level and postgraduate level students as well. Chapter 1 and 2 will give an idea of plants to nanotechnologist group. Chapter 3 to 5 and 10 may help the botanist group to understand the insight of technology while all other chapters are directing both groups to reach to the roots of the link. This book will definitely be changing the vision of botany students.

Lahore, Pakistan Sumera Javad

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Plants and Nanoworld: an Introduction

Nadia Ghaffar and Sumera Javad

1.1 Introduction

This Century ended the difference of basic fields like mathematics, biology, physics etc. and converted them to inter related applied fields like biophysics, biochemistry, biostatistics etc. It paved the way of progress to all the modern technologies available for human beings. These developments in interdisciplinary fields helped scientists in their research and reduced the time of invention magically.

Metal nanoparticles occupy now a basic status in technology as well as in medicines. They are being synthesized by a number of chemical and physical methods. Physical methods include the use of laser and microwaves etc. These methods will be described in detail in Chap. 3 of this book. These methods involve a lot of chemicals, energetic waves, man power and time. There is definitely need of such a method which relies on some renewable source of energy, some natural source with lesser by products as an input for synthesis of nanoparticles. Considering these points we come to ever green treasure of nature i.e. The Plants (Fig. 1.1).

Study of plants is known as Botany. Plants include all flowering (Angiosperms) and nonflowering plants (Gymnosperms, pteridophytes, bryophytes). This is the field of science describing the physiology, ecology, genetics, metabolism, cytology and chemistry of plants on surface of earth. It is one of the oldest knowledge related to science (Fig. 1.2).

Plants grow in all types of environments. They can tolerate extreme environmental conditions like water stress and temperature stress etc. Their physiology and metabolism is changed according to the environment around. Even it has also been reported that different plants produce different types of biochemicals during different seasons i.e., winter and summer. Nature of biochemical produced by plants is also affected by the type of soil in which they are present. It is also dependent upon

N. Ghaffar · S. Javad (✉)
Department of Botany, Lahore College for Women University, Lahore, Pakistan

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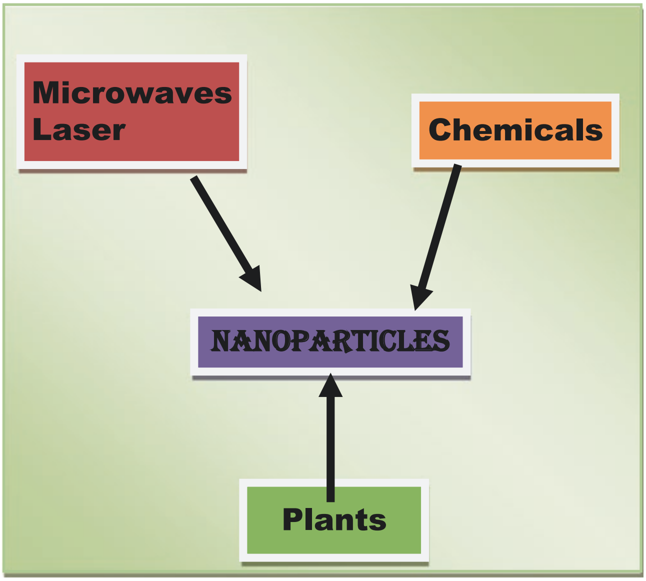

Fig. 1.1 Common sources to synthesize nanoparticles

the genetic makeup of the plant. It means what types of genes are present in that species. So when we talk about the plant based chemicals, we have a tremendous variety and a few of which are described in Chap. 2 of this book.

Nanotechnology is the study of technology of nanoparticles. Nanoparticles are the particles with at least one dimension less than 100 nm. Literature is filled with the information on practical applications of nanoparticles. Researches are now being carried out on development of a sustainable method for the bulk production of nanoparticles. Nanoparticles need some antioxidants or reducing agents whether natural or chemicals or waves, for their synthesis from metal salts and plant metabolites take the responsibility here.

So here arises another practical aspect of applied botany which can be named as "NanoBotany" as it refers to the nano-sized particles synthesized by plants or related to plants. NanoBotany is future of interdisciplinary applied sciences.

1.2 Plant Metabolites

A substance essential to the metabolism of a particular organism or to a particular metabolic process is called metabolite. Human beings have relied upon plants for their necessities like food, shelter and cloths. History shows that they have used different parts of plants for this purpose like leaves, wood, bark, buds, fruits etc. They were also used for arrow poisoning, hallucinations for rituals, poisons for murders and for hunger depressions etc. (Harborne 1984). Nearly 80% of the world's

Fig. 1.2 A Gymnosperm

population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India and Pakistan, majority of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Sandhya et al. 2006).

A plant cell produces two types of metabolites, first are primary metabolites which are produced to target growth and development. Examples of primary metabolites are carbohydrates, proteins and fats while secondary metabolites are produced other than primary metabolites as a byproduct of the plants' main metabolic pathway. They are not basically required for plant growth and development directly. These secondary metabolites don't have any importance in plant classification as they are produced randomly. But only one thing to remember is that they are not junk. They have a purpose in nature. Several secondary metabolites produced in plants play many important functions. For example, terpenoids inhibit competing plants, protect plants from insects and attract pollinators. Alkaloids help plants to resist herbivores and insects. Plant secondary metabolites also help in cellular signaling. Concentrations of theses secondary metabolites may be different in different plants and in different parts of plants but they are playing their role (Bernhoft 2010).

It has also been purposed that plant secondary metabolites are typically defense chemicals. They may be terpenoids, alkaloids, resins or essential oils, they may be specie specific, area specific, family specific but are produced usually in small quantities and it's a difficult job to isolate them (plantscience4u).

These secondary metabolites are now being worked out to be used in nanobiotechnology. They are very good antioxidants naturally and have a great potential to reduce the natural oxidants produced during plant metabolism. They too have a very good potential of forming metal nanoparticles as explained in Chapter no 2 of this book. These may be considered as the very good source of raw material for bulk synthesis of nanoparticles. For practical applications of nanoparticles, they should be of controlled shape and size. It has also been suggested in a number of studies that by controlling the pH, temperature and time of reaction of metal salt with the plant extract.

1.3 Classes and Number of Secondary Metabolites

There is a variety of plant secondary metabolites associated with variety of plants. These secondary metabolites may be specie specific or family specific. Their quantity may vary with environmental stresses. Detail of number of these secondary metabolites is given in Fig. 1.3.

Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups:

1. Terpenoids
2. Nitrogen-containing alkaloids and sulphur containing compounds.
3. Flavonoids and allied phenolic and polyphenolic compounds.

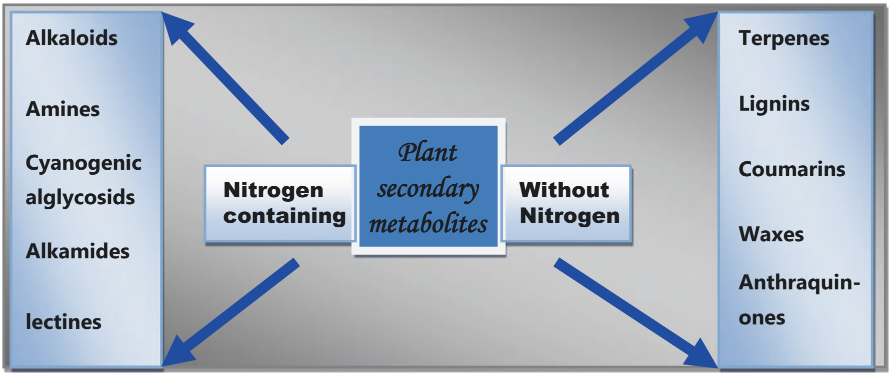

Fig. 1.3 A few types of plant secondary metabolites

1.4 Terpenoids

Terpenoids are the plant secondary metabolites with very diverse nature, like they may be linear in structure or poly cyclic or aromatic. They may be 5-C hemiterpene or having very much complex structure like rubber. But whatever the terpene is, it is made up of the condensation of isoprene units. Isoprene units are formed by the acetate by Mevalonic acid pathway which then join head to head to form different classes of terpens (Mahmoud and Croteau 2002). Terpens are classified by the number of isoprene units they have in their structure.

Some of the common Terpenoids are Steroids, carotenoids, and gibberelic acid, showing its diversity. There are more than 2300 known structures of Terpenoids. They are part of essential oil composition. They are extensively used in the industries, like perfume industry, cosmetics, flavors and spice industry. A number of Terpenoids have medicinal importance having pharmacological activity (Styger et al. 2011).

1.4.1 Mono and Sesqui-terpenoids, and Phenylpropanoids

These are low molecular weight volatile compounds. They are diverse and found among all plant species. Monoterpenes consist of two isoprene units while sesquiterpenes have three units. Phenylpropanoids have less known structures but they form the relative groups consisting of only nine carbons. Their synthetic pathway is also different. These metabolites are lipophilic in nature and are volatile compounds i.e., having low boiling points. They give strong taste and smell. They are extremely used in herbal remedies. Their medicinal uses include antibacterial, antiviral, antifungal and antineoplastic etc. However if these are concentrated then these have toxic effects. The plant family rich in these compounds is *Lamiaceae*, the thyme family. They are also found in other families (Bernhoft 2010).

1.4.2 Diterpenoids

Diterpenoids are consisting of four isoprene units and usually they are not volatile. They don't have any odor. They are lipophilic, so give taste. They have very less known toxocoligical effects. They are found in number of plant families and have very well known medicinal uses (Bernhoft 2010) (Fig. 1.4).

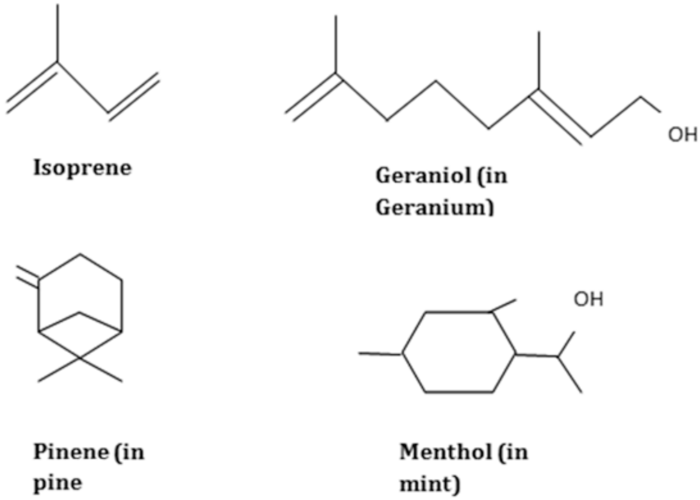

Fig. 1.4 Structure of some terpenoids

1.4.3 Functions

Plants tissues are very much known for their capability to adapt to both biotic and abiotic stresses such as herbivores, pathogens, water and temperature etc. Terpenoids are key to this. However, some terponoids may also affect the surroundings of these plants because they are added to the environment due to their high volatile and reactive nature. Terpenoids also act as infochemicals because they are sensed by the pollinators, neighboring plants and by herbivores, so helping the sessile plants to live. Physiologically function of plant volatiles or terpenoids can be defined in three ways,

1. Plant-plant interaction,
2. The signaling between symbiotic organisms
3. Attracting pollinators i.e., insects.

These roles of terpenoids make them an agricultural tool for pest control to an industrial application for production of flavors and fragrances (Maffei 2010).

1.4.4 Plant Families Rich in Terpenoids

Lamiaceae
Rubiaceae
Cannabaceae
Ginkgoaceae
Lauraceae

1.5 Alkaloids

Alkaloids are basically nitrogen containing heterocyclic compounds, bitter in taste with potent activity. Alkaloids also include some compounds having neutral and weakly acid properties. Besides nitrogen, carbon and hydrogen, alkaloids also contain sulfur, oxygen and rarely some other elements like chlorine, phosphorus and bromine. A clear cut differentiation between alkaloids and nitrogen containing natural compounds is not well known. Some other compounds such as amino acids, amines, peptides, proteins, nucleic acid and nucleotides are not commonly called alkaloids. Their distribution in the plant kingdom is limited but some groups contain various clinical properties (Giweli et al. 2013).

1.5.1 Families Rich in Alkaloids

(i) **Tropane alkaloids** are produced in *Solanaceae* (nightshade family) for example *Hyoscyamus niger* (henbane), *Datura* spp. (thorn apples) and *Atropa belladonna* (deadly nightshade). The compounds usually contain anticholinergic activity (muscarine receptor antagonists) so, used for the treatment of hypersecretion, pain and smooth muscle spasms.

(ii) **Pyrrolizidine alkaloids** are found in *Asteraceae* (daisy family), mostly in *Boraginaceae* (borage family) and *Senecio* spp. (Ragworts). After bioactivation they cause hepatotoxicity in man and animals.

(iii) **Isoquinoline alkaloids:** The main producers of **isoquinoline alkaloids** are *Berberidaceae* (barberry family) and *Papaveraceae* (poppy family). They have biochemical effects and used for medical purpose. They can be used for the inhibition of bacteria, cancer cells and pain, for myocardial contractility, and to stimulate bone marrow leucocytes.

(iv) **methylxanthine alkaloids:** *Theobroma cacao* (cacao) and *Coffea arabica* (coffee) produce **methylxanthine alkaloids**. Binding of Methylxanthines to adenosine receptor causes neurological effects in animals and man, low to moderate intake causes stimulation. High intakes of methylxanthines in rodents elicit reduction in sperm production and testicular atrophy.

(v) **Pseudoalkaloids** are found in families *Apiaceae* (carrot family) and Taxaceae (yew family) for example *Conium maculatum* (hemlock) and *Cicuta virosa* (cowbane); *Taxus baccata* (yew) respectively. Chemical properties of pseudoalkaloids are closely related to alkaloids. *Conium maculatum* and *Cicuta virosa* produce some pseudoalkaloids that greatly effects the central nervous system (Bernhoft 2010).

(vi) **Furocoumarines and naphthodianthrones** Furocoumarines have photosensitizing properties that are mostly produced in *Apiaceae* (carrot family) predominantly in *Heracleum* spp. (cow parsnips). The naphthodianthrones for example in *Polygonaceae* (dock family) e.g. *Fagopyrum esculentum* (buckwheat) and in *Hypericum* spp. (St. John's-worts) of *Clusiaceae* (garcinia family) are similar in action (Fig. 1.5, Table 1.1). Mostly antidepressant effects have been observed in *Hypericum* spp.

1.6 Phenolic Compounds

Plants contain secondary metabolites which have many functional groups. One of them is phenolic group made up of aromatic ring and hydroxyl group. Approximately 8000 different phenolic structures have been studied in plants. Phenols are very diverse in nature and may present in the form of single ring to complexed polyphenols and tannins. Phenols are mostly found attached with carbohydrates and other organic compounds. Classification of these compounds can be done on the number and symmetry of carbon atoms. Common two types of phenolic compounds are flavonoids and non-flavonoids. (Strack 1997) (Fig. 1.6).

1.6.1 Flavonoids

Most common and largest amount of phenolic compounds in the form of polyphenols are flavonoids in plants. These flavonoids are found in epidermis of leaves and fruit's outer covering to protect the plant from diseases, UV absorption, pigmentation and provide stimulus for nitrogen fixing in the nodules of plants. Structurally, flavonoids have 15 carbons and two benzene rings linked with 3 carbon atoms. The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins (Koes et al. 1994).

Some other less common flavonoid groups i.e. coumarins, dihydroflavanols, dihydrochalcones, aurones and flavan-3, 4-diols are also found in our diet. Basic structure of flavonoids has hydroxal group attached to fourth, fifth and seventh carbon. Carbohydrates i.e. glycosides are conjugated with flavonoids are naturally occurring. These glycosides and hydroxyl groups enhance the water solubility of flavonoids. Methayl and isopentyl units in flavonoids made them lipid loving in nature. Flavonols are mostly found in vegetables, fruits and beverages but a difference in their sizes has been seen because of environmental and varietal changes (Crozier et al. 1997).

1.6.2 Flavones

Flavones have two aromatic rings which are attached to heterocyclic pyrene ring and have a base of 15 carbon atoms. Different types of flavones have also been seen i.e. flavonols, flavanone, flavones and many others. Flavonoids are classified into

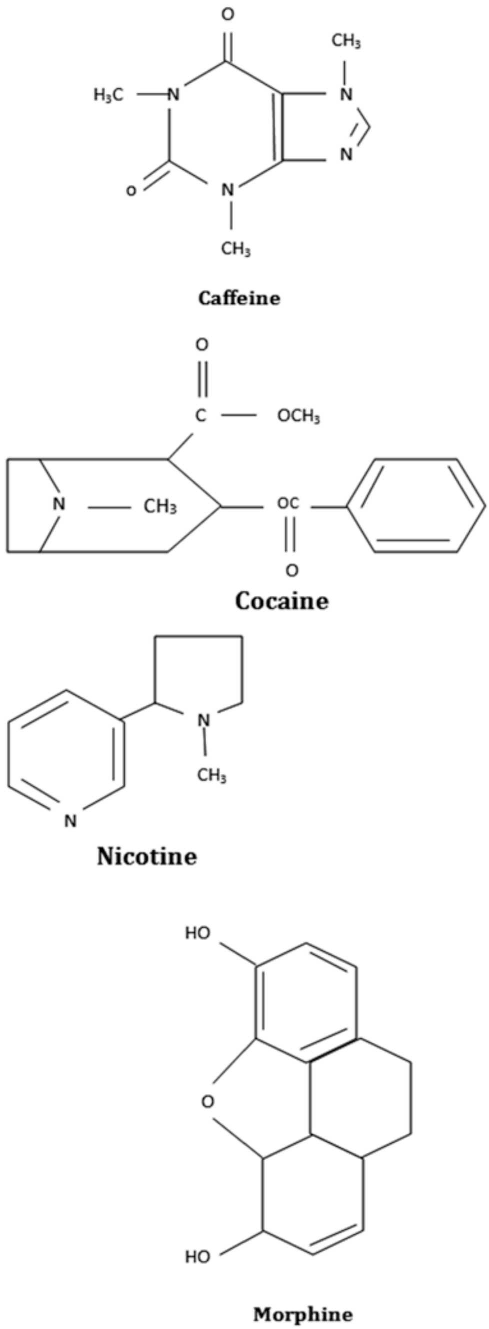

Fig. 1.5 Examples of alkaloids, a diverse group of secondary metabolites that contain nitrogen (Taiz and Zeiger 2010)

Table 1.1 Pharmacological effects of some well known alkaloids

Alkaloid name	Source	Pharmacological activity	References
Nicotine	*Solanaceae* plants family	Stimulant, antiherbivore, insectide, anti inflammatory	Gandhi (2013)
Morphine	*Papaver somniferum* and poppy derivatives	Act on CNS (central nervous system), on myenteric plexus, acute pulmonary edema and reduce the shortness of breath	Rozov-Ung et al. (2014)
Coniine	*Conium macularum, Sarracenia flave*	Neurotoxin, poisonous	Panter et al. (2013)
Atropine	*Atropa belladonna, Darura stramonium, Mandragora officinarum Berberis species, Hydrastis, Canadensis*	Competitive antagonist of muscarinic acetylcholine receptors, anti cholinergic, anti myopia effects	McBrien et al. (2013)

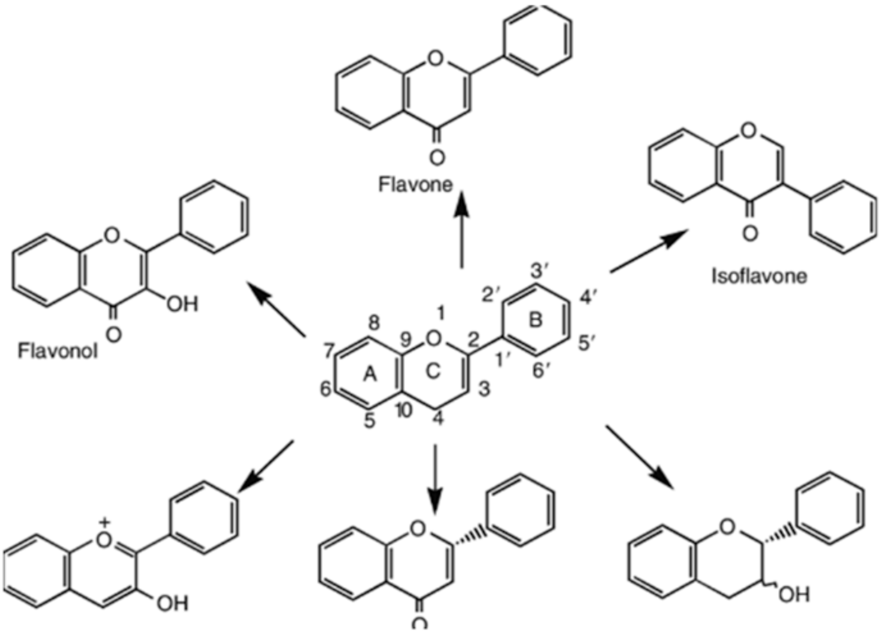

Fig. 1.6 Generic structure of major flavonoid (Anurag et al. 2015)

different classes upon the position of carbon atom and oxidation level whereas compound differences within class is due to pattern of two aromatic rings.

Flavonols are closely associated with flavones in structure. Some Flavones like apigenin and luteolin do not have oxygenation at C_3 but they switch positions with A and C ring.

Flavones have diverse nature for substitution i.e. glycosylation, methylation, alkylation and hydroxylation. Most flavones occur as 7-O-glycosides. Flavones are not much common and found only in significant plants such as parsley, celery and in specific herbs. Some polymethoxylated flavones in citrus plants have also been reported. Some flavones are also associated with diseases such as millet flavones cause goitre in West Africa (Gaitan et al. 1989).

1.6.2.1 Flavan-3-ols

Flavonoids have structurally complex type which include Flavan-3-ols and have simple monomers i.e. (+)-catechin and its isomers to more complexed polymeric condensed form known as tannins. These complexed proanthocyanidins, flavan-3-ols and flavanones are non-planar and have C_3 in heterocyclic ring while other types of flavonoids i.e. tannins, flavonols and many other have planar molecules. Flavan-3-ols have two central chiral carbon atoms C2 and C3 which forms 4 isomers for each step of B-ring hydroxylation. Two of these isomers are (+)-catechin and (−)-epicatechin are common in nature while other two (−)-catechin and (+)-epicatechin are rare (Clifford 1986).

1.6.2.2 Isoflavones

Isoflavones are abundant in leguminaceae especially in soya bean which have 5 main classes of isoflavone. Structure of isoflavone is categorized by the presence of B ring linked to C_3 instead of C_2 position.

1.6.2.3 Plant Families Rich in Phenolic Compounds

Cannabaceae
Ericaceae
Lamiaceae
Pedaliaceae
Rosaceae

1.7 Tannins

Tannin is natural polyphenols and its name derived from a French word Tanin means tanning substance. The tannins are diverse group of oligomers and polymers and they can synthesize complex with minerals, proteins, cellulose and starch. The synthesizes pathway of phenolics such as isoflavons lignins and aromatic amino acids similar to the formation of phenloics and this pathwayis known as shikimic acid pathway, also called as the phenylpropanoid pathway. Tannins are subdivided into two groups based on their solubility in water named as PA (Proanthocyanidins), also