TANNINS & OTHER POLYPHENOLS

Mohammed N. Sabir
Lecture outlines...

- Definition & Introduction...
- Classification...
- Physicochemical properties...
- Isolation from nature...
- Examples and medicinal uses...
- Overview...
TANNINS

Tannins are large phenolic non-nitrogenous biomolecules having astringent properties.

Most are water-soluble, the multiple phenolic hydroxyls and carboxylic groups are responsible for astringency and bitterness.
They are broadly divided into two groups:
1-The hydrolysable tannins, which are formed by the esterification of sugars (e.g. glucose) with gallic acid.
2-The non-hydrolysable tannins, (condensed tannins) occur due to polymerization (condensation) reactions between flavonoids.
Tannins & Flavonoids

Chemical structures of tannins and flavonoids.
Catechin
Isolation

Both hydrolysable and condensed tannins are highly soluble in water and alcohol but insoluble in organic solvents such as ether, chloroform, and benzene.
The general method for the extraction of tannic acid from various galls is either with water-saturated ether, or with mixture of water, alcohol, and ether.
Acetone is added to the extraction procedure to inhibit interaction between tannins and proteins or interfere with the hydrogen bonding between them, thus increasing the yield.
After extraction, the aqueous and ethereal layers are separately concentrated, dried, and subjected to further isolation and purification by chromatography.
The hydrolysable tannins are hydrolysed with bases to simple acids and sugars.
A key feature of tannins is their ability to bind to proteins, and they have been used to tan leather, clarify beer and as astringent preparations in pharmacy.
They have a very wide distribution in plant kingdom and may be produced by a plant as a feeding deterrent, as their binding to proteins may reduce the dietary value of the plant as a food.
Some of these tannins are also plant pigments as anthocyanidins.
Some chemical tests for identification of tannins...

• Reaction with FeCl$_3$.
• Goldbeater's skin test.
• Stiasny's method.
Tannic acid is a mixture of gallic acid esters of glucose and is obtained from nutgall, which is an abnormal tissue growth of the tree *Quercus infectoria* (Fagaceae) as a response of larva inoculation of insects.

*Andricus kollari* مازوزو
The galls are harvested and extracted with solvents (ether and water); the aqueous layer is collected and evaporated to yield tannic acid, which is further purified and used as a topical preparation for cold sores.
Epigallocatechin gallate (EGCG), epigallocatechin-3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin.
EGCG is the most abundant catechin in tea and is a potent antioxidant that have therapeutic applications in (e.g. cancer). [It is found in green tea but not black tea].
In black tea production, the catechins are converted to theaflavins and thearubigins under high temperature, an epimerization change is more likely to occur.
Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41
In addition to antioxidant and antiviral actions, tannins also possess antimicrobial action.
Tannin toxicity for fungi, bacteria and yeasts is due to different mechanisms as been proposed include:-

- Inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth.
- Direct action on microbial metabolism through inhibition of oxidative phosphorylation.
- Iron chelation is also proposed.
Many microorganisms can overcome plant defenses based on tannins through:-

- Synthesis of tannin-complexing polymers.
- Oxidation, tannin biodegradation.
- Synthesis of siderophores.

See the reference...

The most important herbs rich with tannins used in treatment of diarrhoea include:

- *Sangisorba officinalis* L.
- *Acacia catechu* Willd.
- Oak bark
- *Quercus robur* L.
- *Potentilla erecta* (L.) Rausch.
Tannin-containing drugs are generally safe, but care should be taken with concurrent administration of other drugs since tannins are not compatible with alkalis or alkaloids, and form complexes with proteins and amino acids.
Tannins in Herbal Medicine

*Punica garnatum* (Lythraceae)

In the ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years.
General uses of tannins

-Astringent (ppt. of Proteins).

-Hypolipidemic agents.

-Identification of Alkaloids.

-Tan leather.

-In Burns.

-Antidiarrheal agent.

-Antioxidant.
Hamamelis Leaf (Witch hazel leaves)

Dried leaf of *Hamamelis virginiana* (Hamamelidaceae)

- Hama = sametime
- Melis = leaf
Distilled witch hazel extract: prepared by steam hydroalcoholic distillation of the dried leaves and twigs of *H. virginiana*. Contains the volatile oil of the plant.
Presence of tannins in fresh water
Flavonoids and Flavonolignans
- Are the largest group of naturally occurring phenolic compounds

- They occur in different plant parts (fruits, stem, barks, root, leaves, heartwood, flowers)
-They are plant pigments or co-pigments (the yellow color of many fruits)

-They are found in different plant families (Rutaceae, Rosaceae, Astraceae, Lamiaceae, Moraceae, Rubiaceae, and Myrtaceae)
The biosynthesis of flavonoids

I- Phenyl propanoids
   (4-hydroxy coumaric acid)

II- MalonylCoA
Role of flavonoids in plants

1. Insect attractant (pollinating agents)

2. Plant pigments

3. Growth regulators
Role of flavonoids in medicine

- Antioxidants (free radical scavengers)
- Antimicrobial
- Mitochondrial adhesion inhibitors
- Antiulcers
- Estrogen receptor binding
- Cell cycle arrest
- Topoisomerase inhibitors
Examples...

1-Naringin
From *Citrus paradisi*
Fam: Rutaceae
Naringin is an *aldose reductase* inhibitor which means that it can help to fight diabetic retinopathy.
Naringin (and grapefruit) drug interactions

-Interfere

1. Calcium channel blockers
2. Sedatives
3. Cholesterol lowering drugs
4. Caffeine
5. Estrogen

Metabolic interaction through interfering metabolism of these drugs and increasing their t1/2.
Naringin enhance our perception of taste by stimulating the taste buds; that's why some people consume a small amount of grapefruit juice before a meal.
Citrus aurantifolia Peels
Fam: Rutaceae

Uses:
Capillary bleeding (membrane stabilizer)
Flavonolignans

Silymarin (*Silybum marianum* L.)
Milk Thistle
Fam: Astraceae
Silybum marianum

Photos: R. Knox & J. Dodd
Silybin A

Silybin B
Isosilybin A

Isosilybin B
Hepatotoxic agents

• CCl₄
• CHCl₃
• Acetaminophen (Paracetamol©)
• Amanita phalloides
• Xenobiotics
Fatty degeneration in Rat Liver after exposing to CCl$_4$. 

Mechanisms of action of Silybins

1. As antioxidant (inhibit lipid-peroxidation) through free radical scavenging (antioxidant) Key mechanism.

2. Regulators of intracellular content of glutathione.
Mechanisms of action of Silybins

3. Cell membrane stabilizer and permeability regulator that prevent entrance of toxins.

4. Promoters of ribosomal RNA synthesis and stimulating liver regeneration (Hepatocytes protein synthesis).
Mechanisms of action of Silybins

5. Inhibits transformation of stellate hepatocytes into myofibroblasts.

6. Decrease the activity of tumor promoters.

7. Enhances apoptosis.
Water solubility problems of silybin and methods of enhancing its solubility...
Water solubility affects bioavailability and its efficacy...
Bioavailability Enhancement of Silybin Through Carbon-23 Acid Derivatization

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Faculty of Sciences and Health, Koya University, Koya-Iraq.
<table>
<thead>
<tr>
<th>Product</th>
<th>Milk Thistle Herb</th>
<th>Size - Mg.</th>
<th>Silymarin</th>
<th>Silybin</th>
<th>Silymarin / Silybin</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Fruit referred to as seed</td>
<td>one cap/tab</td>
<td>Percent of product</td>
<td>% in mg.</td>
<td></td>
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<tr>
<td><strong>Maximum Milk Thistle</strong></td>
<td>Dried extract (Siliphos)</td>
<td>240 mg.</td>
<td>%33</td>
<td>80 mg.</td>
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<tr>
<td>(Siliphos) (Natural Wellness)</td>
<td></td>
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<tr>
<td><strong>Thisilyn</strong></td>
<td>Dried extract</td>
<td>175 mg.</td>
<td>%80</td>
<td>140 mg.</td>
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<tr>
<td>(Nature's Way)</td>
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<tr>
<td><strong>Legalon</strong></td>
<td>Dried extract (40:1)</td>
<td>90 mg.</td>
<td>%80</td>
<td>70 mg.</td>
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<td>(Madaus, Germany)</td>
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<td><strong>Silymarin 80</strong></td>
<td>Seed Extract plus Milk Thistle seed</td>
<td>210 mg.</td>
<td>%80</td>
<td>168 mg.</td>
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<tr>
<td>(Planetary Formula)</td>
<td>(plus 50 mg.)</td>
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<tr>
<td><strong>Milk Thistle</strong></td>
<td>Seed Extract plus Milk Thistle seed</td>
<td>175 mg.</td>
<td>%80</td>
<td>140 mg.</td>
<td></td>
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<tr>
<td>(Kroeger)</td>
<td>(plus 275 mg.)</td>
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<tr>
<td><strong>Silymarin</strong></td>
<td>Extract (70:1)</td>
<td>210 mg.</td>
<td>%80</td>
<td>168 mg.</td>
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<td>(MediHerb, Australia)</td>
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<tr>
<td><strong>Milk Thistle Seed</strong></td>
<td>Liquid extract (1:1)</td>
<td>35 mg.</td>
<td>%50</td>
<td>17.5 mg.</td>
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<tr>
<td>(Gaia Herbs)</td>
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<tr>
<td><strong>Milk Thistle</strong></td>
<td>Extract</td>
<td>200 mg.</td>
<td>%80</td>
<td>160 mg.</td>
<td></td>
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<tr>
<td>(generic)</td>
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<td><strong>limited information</strong></td>
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NOTES :

-Natural Wellness also has a product called UltraThistle that contains 360 mg of silybin phytosome per capsule.

-Thisilyn (distributed in the United States) uses the same ingredients as Legalon (Germany).

-Silymarin 80 contains 50 mg of organically grown and processed milk thistle seed.

-Siliphos and Phytosome are registered trademarks of Indena (Italy).

-Silybin Phytosome is also available from Enzymatic Therapy – 120 mg per capsule.
Lecture overview...
Thanks for listening...

Any questions?