Chapter

3

Phenylpropanoids and Flavonoids

Phenylpropanoids

The phenylpropanoids are organic compounds that are biosynthesized by plants from the amino acids phenylalanine and tyrosine in the shikimic acid pathway. Their name is derived from the six-carbon, aromatic phenyl group and the three-carbon propene tail of coumaric acid, which is the central intermediate in phenylpropanoid biosynthesis. The biosynthesis of myriad natural products including lignin, lignocellulose, flavonoids, isoflavonoids, coumarins, aurones, stilbenes, catechin, and phenylpropanoids proceeds from 4-coumaroyl-CoA. The coumaroyl component is produced from cinnamic acid.

This non-terpenoid group is obtained from n-propyl benzene. The aromatic ring may contain methoxy, methylenedioxy groups, and hydroxy groups. The propyl side chain may contain carboxylic or hydroxy group. Phenylpropenes contain subfamily of phenylpropanoids. Examples of such phenylpropanoids are methyl chavicol, trans-anethole, isoeugenol, eugenol, vanillin, myristicin, cinnamaldehyde, and safrole.

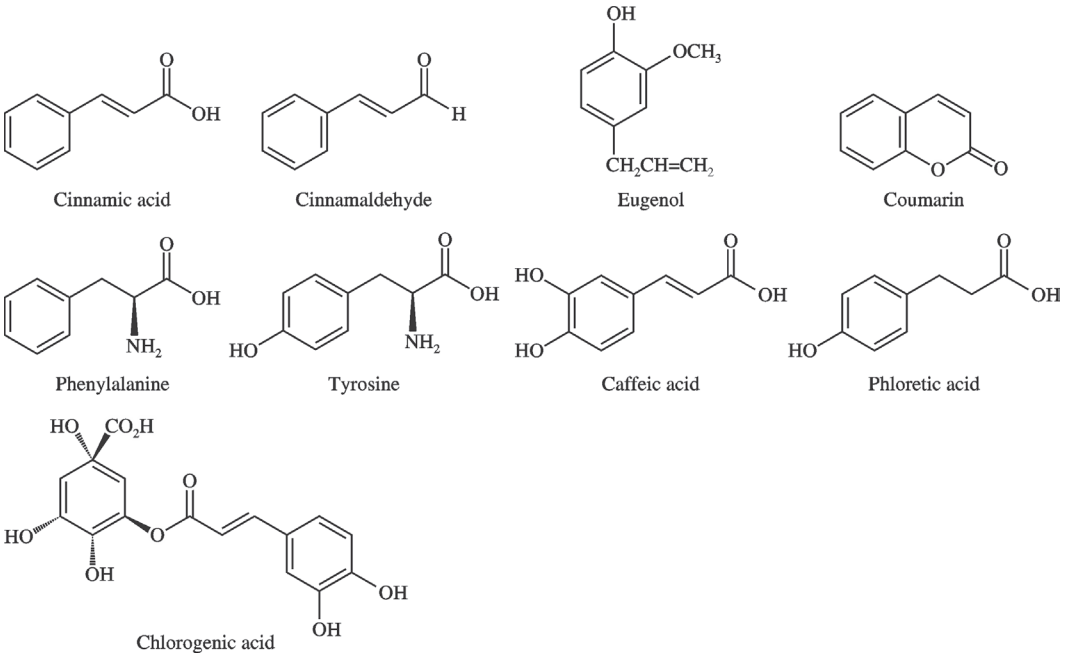
A major of phenylpropanoids is phenylalanine, but other carbon sources, e.g. tyrosine also contribute. The elimination of ammonia from phenylalanine by phenylalanine ammonia lyase (PAL) enzyme gives cinnamic acids. This group of compounds occurs widely in the plant kingdom. Two mono-hydroxy derivatives of cinnamic acid, 3-hydroxycinnamic acid and 4-hydroxycinnamic acids have been found together with caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid, 5-*O*-caffeoylquininc acid (chlorogenic acid) and 3-(4-hydroxyphenyl) propanoic acid (phloretic acid).

The phenylpropanoid pathway provides the building blocks for lignin, suberin, and condensed tannins that play a role in structural support and mechanical strength. Phenylpropanoids are found throughout the plant kingdom. They serve plants as essential components of a number of structural polymers, provide protection from ultraviolet light, defend against herbivores and pathogens, and mediate plant-pollinator interactions as floral pigments and scent compounds.

Phenylpropanoids and other phenolics are part of the chemical composition of sporopollenin. It is related to cutin (a waxy polymer of the plant cuticle) and suberin. This substance found in pollen provides resistant to degradation. It is a mixture of biopolymers, containing mainly hydroxylated fatty acid, phenylpropanoids, phenolics and traces of carotenoids.

Phenylpropanoids are economically important metabolites. They constitute important components in the human diet, acting as nutraceutical compounds with antioxidant, chemopreventive, antimitotic, neuroprotective, cardioprotective, and anti-inflammatory activities. Several phenylpropanoids are considered high-value biochemicals used in the production of fragrances, pharmaceuticals and biopolymers.

The structural diversity of phenylpropanoids is due to a variety of further enzymatic transformations, including acylation, condensation, cyclization, glycosylation, hydroxylation, methylation, and prenylation. Structures of some of procurers of Phenylpropanoids are given hereunder:



Flavonoids

Flavonoids are polyphenolic secondary metabolites found in plants. They chemically have the general structure of a 15-carbon skeleton, which consists of two phenyl rings A and B) and an oxygen embedded in the heterocyclic ring. Ring A usually shows a phloroglucinol substitution pattern. This carbon structure can be abbreviated C6-C3-C6.

Isoflavonoids are derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure. Neoflavonoids are derived from 4-phenylcoumarin (4-phenyl-1,2-benzopyrone) structure. The flavonoids, isoflavonoids and neoflavonoids are all ketone-containing compounds. For example, anthoxanthins (flavones and flavonols) contain ketone group at C-4 position.

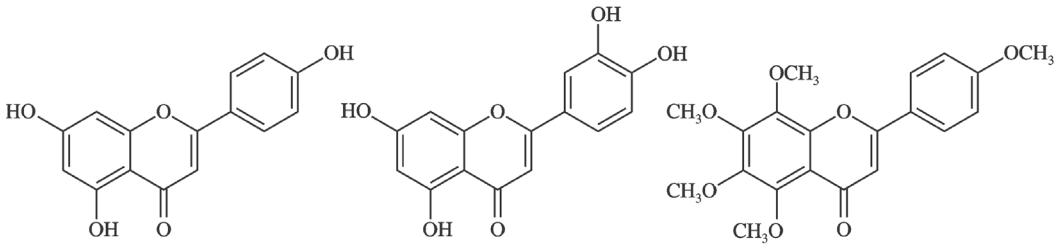
Depending on the chemical structure, degree of oxidation, and unsaturation of the linking chain (C3), flavonoids can be classified into different groups, such as flavones, flavonols, isoflavones, flavanones, flavan-3-ols, flavanonols, and anthocyanidins. Chalcones are lacking the heterocyclic ring. They are also classified as flavonoids. Furthermore, flavonoids can be found in plants in glycoside-bound and free aglycone forms. The glycoside-bound form is the most common flavone and flavonol form consumed in the diet.

Flavonoids are the most important plant pigments for flower coloration, producing yellow, red, or blue pigmentation in petals designed to attract pollinator animals. In higher plants, they filter the UV radiation, symbiotic nitrogen fixation, and floral pigmentation. They also act as chemical messengers, physiological regulators, and cell cycle inhibitors.

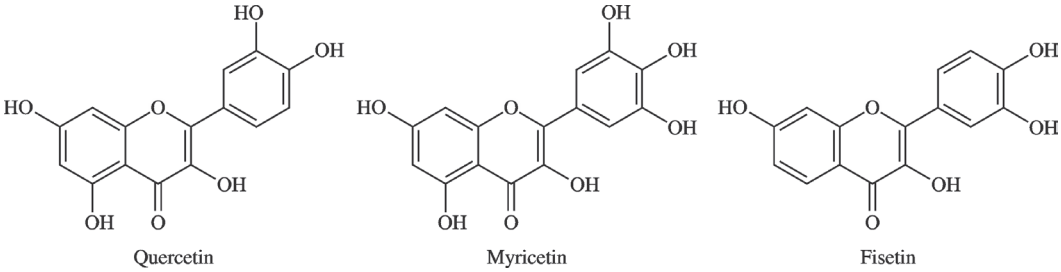
Classification: Over 5000 naturally occurring flavonoids have been characterized from various plants. They have been classified according to their chemical structure, and are usually subdivided into the following subgroups:

Flavones: Flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Common flavones include apigenin (4′,5,7-trihydroxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone), tangeritin (4′,5,6,7, 8-pentamethoxyflavone), chrysin (5,7-dihydroxyflavone), and 6-hydroxyflavone.

Flavones are common in foods, mainly from spices, and some yellow or orange fruits and vegetables, e.g. in parsley, red peppers, celery, chamomile, and peppermint. They are the pigments in blue and white flowering plants. They are natural pesticides, protecting leaves from harmful insects. Flavones may also help with inflammation in the body.

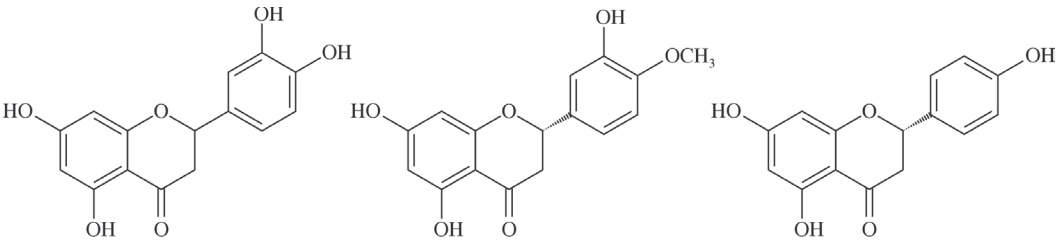


Flavonols: Flavonols have the 3-hydroxyflavone backbone (IUPAC name: 3-hydroxy-2-phenylchromen-4-one). Their diversity stems from the different positions of the phenolic hydroxy groups. The flavonols are quercetin, myricetin, and fisetin. Flavonols are linked to many health benefits, including a lower risk of heart disease. Flavonols are present in a wide variety of fruits and vegetables. They are found in lettuce, tomatoes, onions, kale, apples, grapes, berries, tea, red wine, peaches, berries, scallions, and broccoli.



Flavanones: The flavanones are various aromatic, colorless ketones derived from flavone that often occur in plants as glycosides. Flavanones include Blumeatin, Butin, Eriodictyol, Hesperetin, Hesperidin, Isosakuranetin, Naringenin, Pinocembrin, Pinostronin, Poncirin, Sakuranetin, Sakuranetin, and Sterubin.

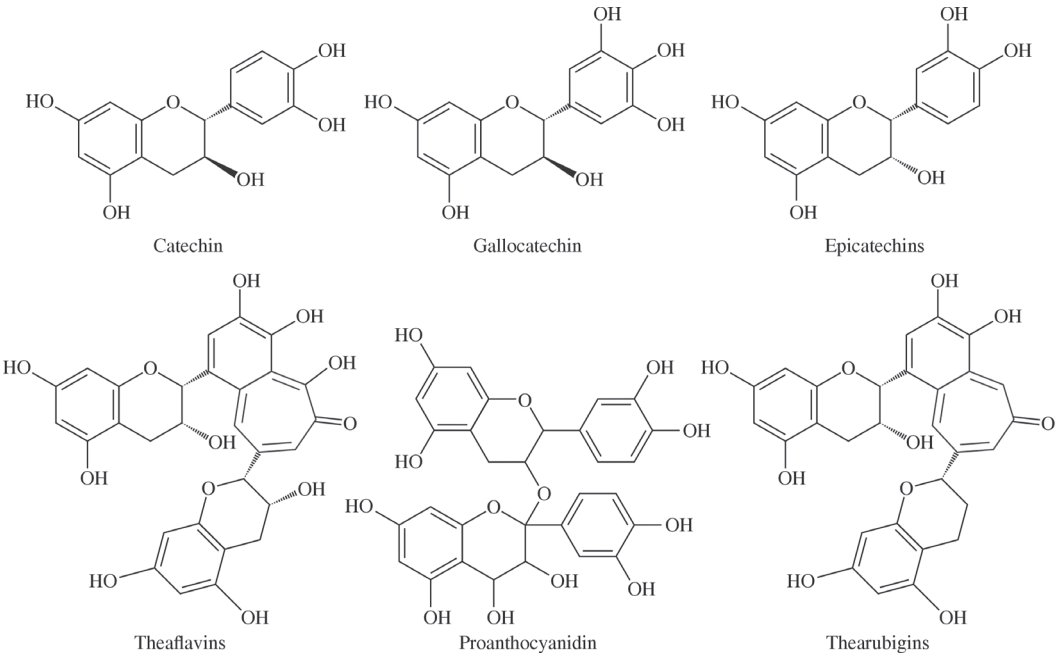
Citrus fruits like oranges, lemons, limes, and grapefruits have flavanones. These compounds make citrus juice and peels taste bitter. They have anti-inflammatory property. They help to manage body weight and cholesterol.



Flavan-3-ols: Flavan-3-ols, also called flavanols or catechins, are derivatives of flavans that possess a 2-phenyl-3,4-dihydro-2*H*-chromen-3-ol skeleton. Flavanols include Catechin, Gallocatechin, Catechin 3-gallate, Gallocatechin 3-gallate, Epicatechins, Epigallocatechin, Epicatechin 3-gallate, Proanthocyanidins, Theaflavins, thearubigins, and Epigallocatechin 3-gallate. Flavanols are present in black tea, oolong tea, and chocolate, and in fruits, including bananas, blueberries, peaches, apples, pears, purple and red grapes, blueberries, strawberries, cocoa and chocolate products. They play a part in plant defence system.

Proanthocyanidins are dimers, trimers, oligomers, or polymers of the flavanols.

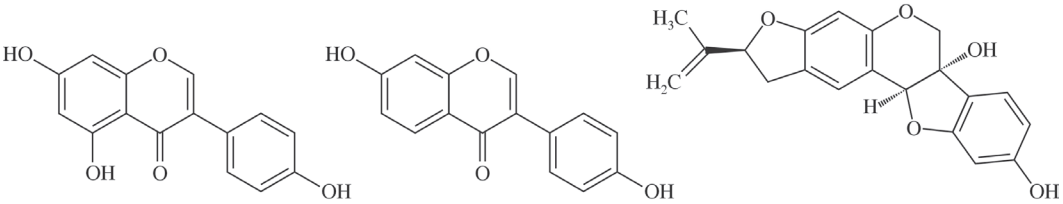
Theaflavins include Theaflavin-3-gallate, Theaflavin-3′-gallate, and Theaflavin-3, 3′-digallate.



Isoflavonoids: Isoflavonoids have the 3-phenylchromen-4-one skeleton with no hydroxyl group substitution on carbon-2 position. The examples include Genistein, Daidzein, and Glycitein. Isoflavones help to keep hormones balanced in the body. Isoflavonoids are mainly present in soy, soy products, and some other legumes such as fava beans. Medically, isoflavonoids have been used in many dietary supplements. Recently, some

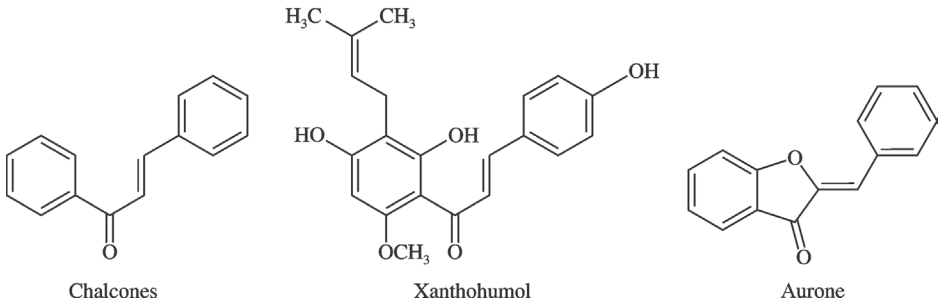
natural isoflavonoids have been identified as toxins, including biliatresone which may cause biliary atresia when infants are exposed to the plant product.

The isoflavonoid group is broad, and includes many structurally similar groups, e.g. isoflavones, isoflavanones, isoflavans, pterocarpans, and rotenoids. Flavonoids have the 2-phenylchromen-4-one backbone, but isoflavonoids have the 3-phenylchromen-4-one backbone with no hydroxyl group substitution at position C-2.



Chalcones: A chalcone is the organic compound, $C_6H_5C(O)CH=CHC_6H_5$. It is an α, β-unsaturated ketone. They are widely known as bioactive substances, fluorescent materials, and chemical intermediates. These compounds have antioxidant properties. Some types of these compounds are used in beauty products. Foods high in chalcones include Tomatoes, Pears, Strawberries, Bearberries, and Wheat. Xanthohumol is also a chalcone.

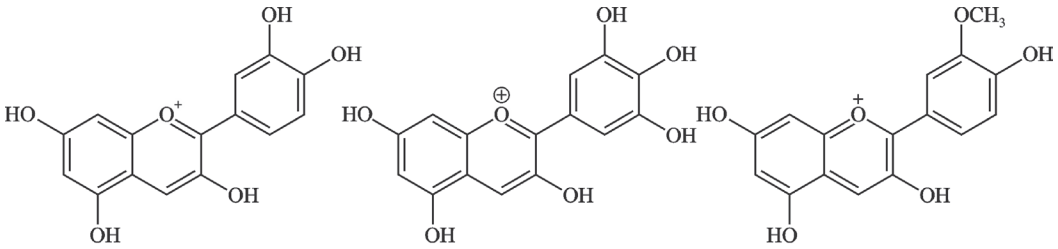
In medicinal chemistry, chalcones are used as antioxidant, anticancer, antidiabetic, antiviral and antimalarial drugs. In chemical industries, they are used as liquid crystals, fluorescent chemical scaffolds, metal sensors, corrosion inhibitors and plant hormones. Chalcones are used as intermediates in heterocyclic synthesis, especially in the synthesis of aurones and pyrazoles.



Anthocyanidins: Anthocyanidins are common plant pigments. They are the aglycones of anthocyanins. They are based on the flavylium cation (2-phenylchromenylium) ion skeleton with various groups substituted for its hydrogen atoms. Anthocyanidins are the aglycones of anthocyanins. The examples are Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin and Petunidin. They generally change color from red through purple, blue, and bluish green due to change of pH values. The color of red, purple, and blue fruits comes from anthocyanins. They are found in Red grapes, Cranberries, Raspberries, Strawberries, Blueberries, Bilberries, and Blackberries.

At least 31 monomeric anthocyanidins have been properly identified in living organisms, mostly as the core components of anthocyanins. The latter are responsible

for the red, purple, blue, or black color of many fruits (like grapes and blueberries), flowers (like rose), leaves (like purple cabbage), and even tubers (like radishes and purple yams). They are also found in some animals.



Dietary sources: Flavonoids such as the catechins are the most common group of polyphenolic compounds in the human diet. Flavonols, e.g. quercetin, are very common and found everywhere, but in lesser quantities. Many animals, including humans, ingest significant quantities of flavonoids in their diet. Foods with a high flavonoid content include parsley, onions, blueberries and other berries, black tea, green tea, oolong tea, bananas, all citrus fruits, *Ginkgo biloba*, red wine, sea-buckthorns, buckwheat, and dark chocolate with a cocoa content of 70% or greater. Flavonoids have antioxidant properties and may lower the risk of heart attack or stroke.

Anthocyanins are naturally produced pigments that give flowers their red, purple, and blue color. They are predominantly found in the outer skin of berries and berry products like red and purple grapes, red wine, cranberries, blueberries, strawberries, and blackberries.

Flavonoid Tests for Detection

Shinoda test: Four pieces of magnesium filings are added to the ethanolic extract followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids. Colours varying from orange to red showed the presence of flavones, red to crimson indicated flavonoids, crimson to magenta colour is due to flavonones.

Sodium hydroxide test: About 5 mg of the compound is dissolved in water, warmed, and filtered. An aqueous sodium hydroxide (10%) is added to 2 ml of this solution. This produces a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid is an indication for the presence of flavonoids.

p-Dimethylaminocinnamaldehyde test: A colorimetric assay based upon the reaction of α-rings with the chromogen p-Dimethylaminocinnamaldehyde has been developed for flavonoids in beer that can be compared with the vanillin procedure.

Health benefits of flavonoids: Flavonoids help regulate cellular activity and fight off free radicals that cause oxidative stress on the body. In simpler terms, they help the body function more efficiently while protecting it against everyday toxins and stressors.

Antioxidant effects: Flavonoids are also powerful antioxidant agents. The human body makes free radicals, unstable molecules that can damage cells. This damage can lead to inflammation and contribute to other problems such as cancer and heart disease.

Antioxidants help neutralize free radicals. Flavonoids have been shown to have antioxidant effects. They might help to prevent chronic diseases, but the body may not absorb them as well as other antioxidants like vitamin C.

Heart disease prevention: Flavonoids might reduce your risk for heart disease. Their antioxidant activity could help lower inflammation and blood pressure.

Diabetes prevention: Flavonoids may reduce risk for type 2 diabetes because they improve how the body uses glucose (sugar) and digests carbohydrates. A person that consume lots of flavonoids has a lower risk of diabetes.

Brain health: Flavonoids might protect the brain by lowering inflammation and protecting the vascular (blood vessel) system. Cocoa flavonoids could improve brain function, memory, and blood flow.

Cancer prevention: A diet rich in flavonoids leads to a reduced risk of breast, prostate, and colorectal cancers. Different flavonoids protect against specific types of cancer. For example, anthocyanidins decrease lung cancer risk, while flavonols reduce the risk of prostate cancer.

Managing chronic pain and inflammation: Flavonoids reduce cells' response to pain. They are used to manage chronic pain and treat inflammatory diseases.

Inflammation is one of the body's immune responses. Allergens, germs, toxins, and other irritants can trigger inflammation that results in uncomfortable symptoms. Flavonoids may help the body to dismiss that inflammatory reaction so that those symptoms are reduced.

Treating viral infections: Flavonoids have known antibacterial and antiviral effects. Certain flavonoids can help keep the H1N1 flu, HIV, SARS, and RSV viruses from reproducing themselves.

Lignans

Biological source: The lignans are fibre-associated low molecular weight polyphenols found in plants, particularly oil seeds, whole grains, fruits, legumes and vegetables. Lignans are precursors to phytoestrogens. They play a role as antifeedants in the defence of seeds and plants against herbivores. The highest concentrations of dietary lignans are found in flaxseed as secoisolariciresinol diglucoside.

Lignans are phenolic dimers possessing a 2,3-dibenzylbutane structure. They exist as minor constituents of many plants, where they form the building blocks for the formation of lignin in the plant cell wall. The compounds occur mainly in the glycosidic form. The two major mammalian lignans, enterodiol and enterolactone, are the products of colonic bacterial metabolism of the plant lignans secoisolariciresinol and matairesinol.

Lignans are also known as phytoestrogens, found in plants. Plant dietary lignans such as pinoresinol, lericiresinol, secoisolariciresinol, syringaresinol, or sesamin are derived from phenylanaline. Typically, lariciresinol and pinoresinol contribute about 75% to the total lignan intake, whereas secoisolariciresinol and matairesinol contribute only about 25%.

Flax seeds and sesame seeds contain high levels of lignans. The principal lignan precursor found in flaxseeds is secoisolariciresinol diglucoside. Other foods containing lignans include cereals (rye, wheat, oat and barley), soybeans, tofu, cruciferous vegetables, such as broccoli and cabbage, and some fruits, particularly apricots and

strawberries. Lignans are not present in seed oil, and their contents in whole or ground seeds may vary according to geographic location, climate, and maturity of the seed crop, and duration of seed storage.

Furofuran lignans have been reported in 27 plant families, including mainly Thymelaeaceae, Styracaceae, Scrophulariaceae, Saururaceae, Rutaceae, Piperaceae, Pedaliaceae, Lauraceae, Lamiaceae, Compositae, Arecaceae, Araliaceae, Apocynaceae, Acoraceae and Acanthaceae. Furofuran lignans are present in the bark, bulbs, leaves, seeds, stems and roots of these plants.

Lignans are bioactive compounds exhibiting various biological properties, including anti-inflammatory, antioxidant and antitumor activities.

The highest lignan content is observed in beverages, such as tea, coffee, red wine and white wine.

The chief source of dietary fat in Mediterranean countries is extra virgin olive oil (EVOO). Lignan sources in the diet of a Mediterranean population included garlic, onions, vegetables, grains and seasonal fruits, including citrus.

The typical Indian diet include fish, grapes, chocolate, oils, coffee, tea, biscuits and bread. The fruit of *Morinda citrifolia* (Indian mulberry) are utilized to treat cancer, diabetes, high blood pressure, diarrhea, headache and inflammation, due to its high lignan content.

Many major plant sources of lignans in China are also used as medicinal plants, e.g. *Articum lappa*. Its fruit and seeds are a rich source of bioactive lignans, including arctiin and arctigenin. These two lignans exhibit anti-inflammatory activitys. Some species, such as *Isodon japonica*, have been used in traditional Chinese medicine to treat arthralgia, stomach-ache, mastitis, gastritis and hepatitis. *Isodon rubescens* is used in traditional medicine for its hypotensive, antioxidant, immunological, antimicrobial, antitumor and anti-inflammatory properties.

Lignans can be divided into eight structural subgroups, e.g. (1) dibenzylbutyrolactol (2) dibenzocyclooctadiene (3) dibenzylbutyrolactone (4) dibenzylbutane (5) arylnaphthalene (6) aryltetralin (7) furan and (8) furofuran. Each subgroup can be further subdivided according to lignan molecule oxidation level and identities of non-propyl aromatic rings present on side chains.

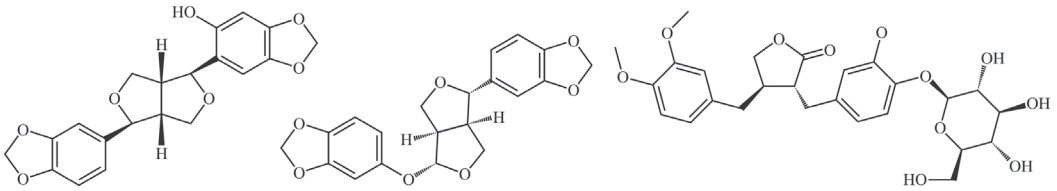
Chemical constituents: Lignans are diphenolic compounds formed of two phenylpropane units. Several lignans, such as secoisolariciresinol, are considered as phytoestrogens.

The sesame seeds contains sesame lignans, such as (+)-sesaminol, (+)-sesamolin, sesamin, sesamol, and (+)-sesamin glucosides. They showed anti-inflammatory, antioxidant and anti-hypertensive activities.

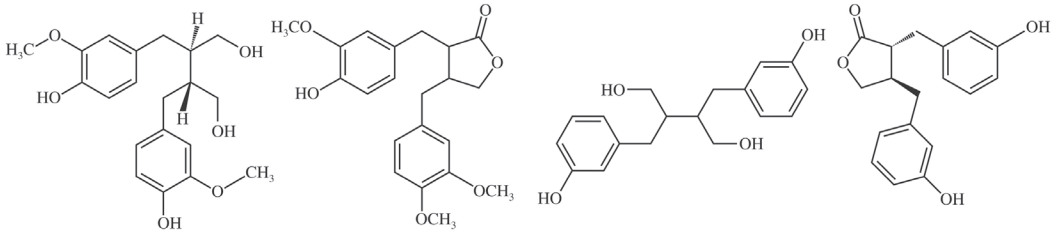
Articum lappa, whose fruit extracts and seeds are a rich source of bioactive lignans, including arctiin and arctigenin.

Linum usitatissimum L. (flaxseed) is one of the richest sources of lignans, followed by cereals and legumes. It contains 800 times more lignans and norlignans than other medicinal plants. Secoisolariciresinol diglucoside, matairesinol, secoisolariciresinol and lariciresinol diglucoside are the most abundant lignans produced by flax seeds.

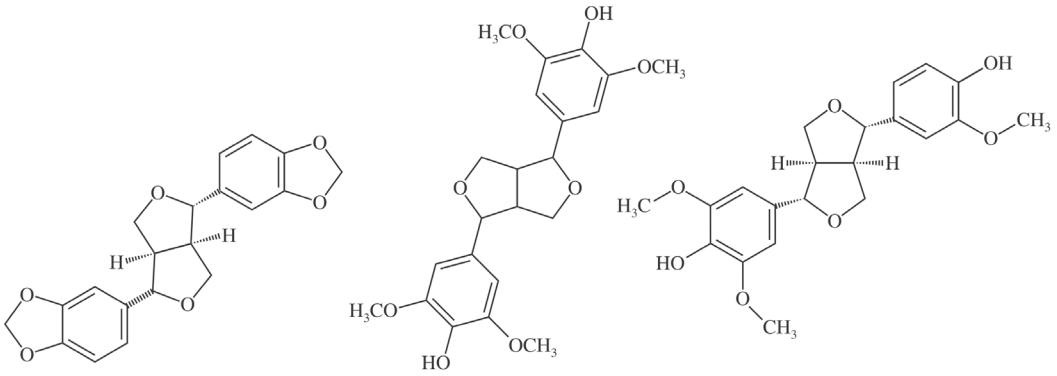
The major lignans, which possess numerous pharmacological properties, are arctigenin, enterodiol, enterolactone, sesamin, syringaresinol, medioresinol, (−)-matairesinol, (−)-secoisolariciresinol, (+)-lariciresinol and (+)-pinoresinol. Podophyllotoxin is isolated from the rhizome resin of several *Podophyllum* species.



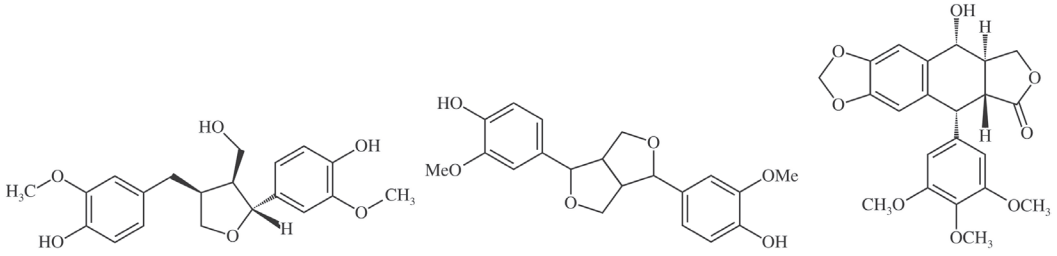
Sesaminol Sesamolin Arctiin



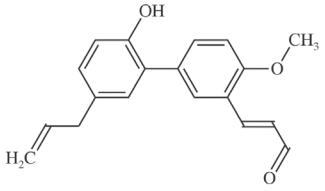
Secoisolariciresinol Arctigenin Enterodiol Enterolactone



Sesamin Syringaresinol Medioresinol



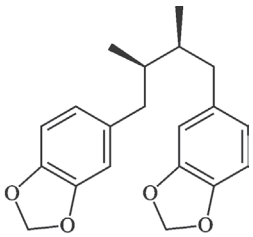
Lariciresinol Pinoresinol Podophyllotoxin



4-Methoxymagnaldehyde B

4-Methoxymagnaldehyde B and coumanolignan, 4′-methoxymagndialdehyde, 4′-methoxymagnaldehyde B, and 4′-methoxymagnaldehyde E are the lignans isolated from the stem bark of *Magnolia obovata*.

The bark of *Machilus thunbergii* from Izu Peninsula contains simple lignans machilin A, *meso*-dihydroguaiaretic acid, *meso*-austrobailignan-6 and *meso*-monomethyl dihydroguaiaretic acid.


Machilin A

Nectandrin A, and nectandrin B are 7,7′-epoxylignans present in the leaves and stems of *Nectandra rigida* Nees and *Machilus thunbergii*.

(–)-Parabenzoinol is a 7,9′-epoxylignans was isolated from the leaves of *Parabenzoin trilobum* Nakai.

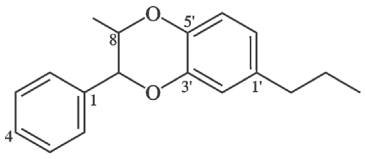
(–)-Parabenzlactone and acetylparabenzylactone are lignan-9,9′-olides found in the leaves of *P. trilobum* Nakai. 5,6-Dihydroxymatairesinol occurs in the stems of *Lindera obtusiloba*.

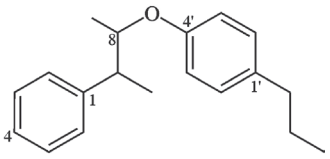
(–)-Isoguaiacin and (+)-guaiacin are 2,7′-cyclolignans isolated from the bark of *Machilus thunbergii*. (+)-Otobaphenol was isolated from *Psidium cinereum*, called katuaba, a species of plant in the family Myrtaceae, endemic to Brazil. Cinnamophilin A was obtained from the roots of *Cinnamomum philippinense* (Merr.) Chang. (–)-Aristoligone, (–)-aristotetralone, and (–)-cagayanone A were obtained from the leaves and twigs of *L. chinpingensis*.

Neolignans are widely distributed in the Lauraceae family, especially in the genera of *Aniba*, *Nectandra*, and *Ocotea.* The types of neolignans isolated from Lauraceae include 8,1′-neolignans (from *Aniba burchellii*), 8,3′-neolignans (from *Nectandra glabrescens*), 7,1′-neolignans (from *Licaria chrysophylla*), and 7,3′-neolignan, and 3, 3′-neolignans.

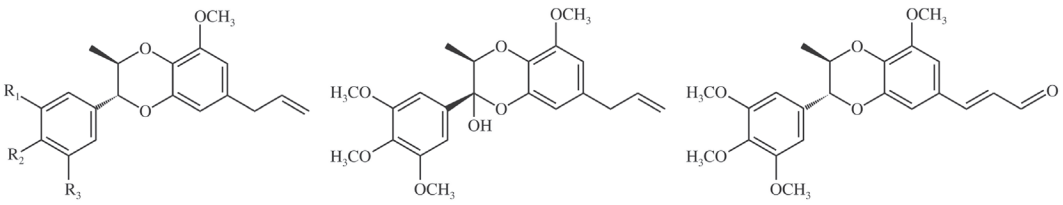
Chrysophyllon II-A and chrysophyllon II-B belonging to the category of 7,3′-neolignans, both were found to occur in the bark, wood, and fruit calyces of *Licaria chrysophylla*.

An ether oxygen atom provides the linkage between the two phenylpropane units, giving rise to oxyneolignans. Oxyneolignans are rarely distributed in Lauraceae.

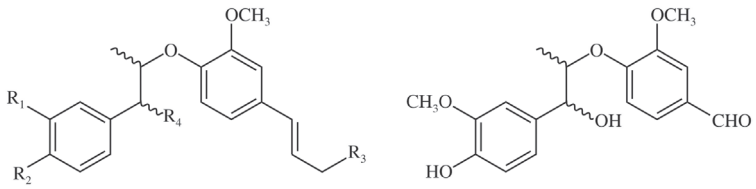

7.3',8.4'-dioxyneolignan


8,4'-oxyneolignan

The trunk wood of *Licaria* rigida (Lauraceae) contained 7.3′,8.4′-dioxyneolignans eusiderin A and eusiderin B. Eusiderin was also present in the ethanolic extract of wood of *Oedopeza costulatum*.



Machilin C, D, and E are 8,4′-Oxyneolignans obtained from the bark of *Machilus thunbergii*. Odoratisol B was obtained from the air-dried bark of the Vietnamese medicinal plant *M odoratissima* Nees.



Chemical structures of 8,4′-oxyneolignans

Extraction of lignans: The extraction processes include the choice of extraction solvent, method, time, temperature, solvent-to-sample ratio, and the number of repeat extractions of a sample.

Solvents: For plant material containing aglycones, medium polarity solvents, such as ethyl acetate. For the lignan glycosides aqueous mixtures of ethanol and methanol give the best results. In the case of very polar lignan glycosides, pure water may be used.

Methods: Extraction of Lignans can be used the conventional procedures such as Soxhlet, maceration, and digestion, were applied.

Contrary to conventional methods, maceration is performed at room temperature. It is mainly used to extract thermolabile compounds. Although macerations for 1 h have been performed for the extraction of lignans, they usually require much longer extraction times, reaching up to 7 days.

Soxhlet and other heated reflux techniques are the most common methods used in lignans' extraction. The temperature usually is performed at 80–100 °C, a temperature capable of boiling ethanol, methanol, and their dilutions, other organic solvents and water. The main advantage of this method is limiting the use of large volumes of solvents, as one batch of solvent is constantly recycled. The time of extraction under this method varies from the 30 min used in the extraction of eleutheroside E (syringaresinol diglucoside) from *Eleutherococcus senticosus* root, up to 10 h for the extraction of sesamin and sesamolin from the sesame seeds.

Ultrasound-assisted extraction (UAE) was used in the recovery of lignans from *Linum usitatissimum* L. seeds, *Sesamum indicum* L. seeds, *Arctium lappa* L. fruit, and Forsythia species fruits, stems, and roots.

Another important method is microwave-assisted extraction (MAE). About 70% methanol dilution was chosen as the best extraction solvent, 10 min as the extraction time, and 1:30 as the sample-to-solvent ratio.

Lastly, supercritical fluid extraction (SFE) may be used as another extraction technique. It can lower the use of extraction solvents, shorten extraction time, and increase the yield.

Isolation and purification: The separation of individual structures is an important step for the chemical analysis of the raw material. The obtained pure compounds can be valuable as new standards for HPLC-MS analysis and for biological experiments to determine their activities.

Flash chromatography on silica gel or Sephadex LH-20 columns has been used for the initial fractionation, separation, purification and preparative isolation of natural compounds from raw materials. In addition, various chromatographic methods, such as

open column chromatography, medium performance liquid chromatography (MPLC), and semi-preparative HPLC may be used.

Liquid chromatography is a bidirectional technique for isolating and purifying a single compound from a mixture of many substances, often having very similar molecular structures. The preparative chromatography can be divided into several subgroups, for example, semi-preparative chromatography for the isolation of a few micrograms of compound usually for the assessment of biological properties, laboratory-scale preparative chromatography for the isolation of a few to several grams of analyte as an intermediate in a synthesis process, or for detailed studies of the pharmacological and pharmacokinetic mechanisms.

Using a semi-preparative HPLC system equipped with a UV–VIS detector is the most versatile method to obtain pure the lignans with UV absorption monitored at 280 nm or 254 nm. It can be conducted on a reverse-phase C18 column using an isocratic elution, as was presented during noreucol A, (+)-epiolivil, or (+)-olivil isolation from Eucomiae bark, lignans from *Euphorbia hirta* L., and neolignans from *Pouzolzia sanguinea* (Blume) Merr. The isolation of lignans was performed from the seeds of *Arctium lappa* L. obtaining arctigenin, matairesinol, arctiin, and a mixture of two isomers containing lappaol A and isolappaol A, using preparative and semipreparative chromatography.

Qualitative and quantitative analyses: Thin Layer Chromatography (TLC) has been applied as an inexpensive supporting method in lignan analysis, e.g. during the qualitative examination of plant extracts, fraction collection, monitoring isolation procedures, or the screening of many samples.

In lignan TLC analysis, silica gel is mostly used, with different eluents and detection methods. The choice of eluent, similar to the extraction solvent consideration, depends on the physicochemical properties of the lignans present in the sample.

The solvent system dichloromethane: ethanol 93:7 (v/v) is used for samples containing aglycones from conifer wood. The samples from seeds of *Carthamus tinctorius* L., containing glucosides of trachelogenin, arctigenin, and matairesinol, are analysed using with toluene: ethyl acetate: formic acid 3:1:0.2 (v/v/v) and dichloromethane: acetone:formic acid 7:2:0.1 (v/v/v).

Various detection techniques have been applied for lignans. As all lignans absorb UV light, plates without chemical treatment can be observed under 254 nm wavelength.

The High-Performance Thin Layer Chromatography (HPTLC) method is used to quantify sesamin in sesame oil without saponification, which confirms the validity of this method in lignans' analysis.

High-performance liquid chromatography (HPLC): This is a technique of choice applied for the detection, identification, separation, and quantification of lignans from plant matrixes or human fluids. Based on the literature data, analysis of the lignans was performed by HPLC with various detection methods, including UV detection, coulometric electrode array detection (CEAD), pulse amperometric detection (PAD), fluorescence detector (FLD), and mass spectrometry (MS). In most cases, the analyses are performed with satisfying sensitivity in the UV detection at 280 nm or 254 nm.

Liquid chromatography mass spectrometry: The liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS) has provided a routine method for the identification of plant metabolites (including lignans) in complex plant matrixes. In most cases, the compounds are identified by comparing fragmentation patterns with available libraries or using reference compounds.

Gas chromatography mass spectrometry: Gas chromatography (GC) is a less popular method for the identification of phenolic compounds from the lignan group due to their low volatility and the need for high temperatures, which can damage the analytes. This method allows the identification of multicomponent mixtures (e.g. polyphenols), combined with a detection system (usually a mass spectrometer—GC-MS) giving unambiguous information about its composition, and thus can be an alternative to LC-MS.

Therapeutic uses: Lignans are used to cure lowered risk of heart disease, menopausal symptoms, osteoporosis and breast cancer. They lower the risk of cancer, reduce hot flashes in postmenopausal women, level of bad cholesterol, and free radical damage to cells, protect heart health, clean the gut and help bowel movement, ovarian and uterine health in women, and prostrate health in men.

Flaxseed oil, fibers and flax lignans have potential health benefits such as in reduction of cardiovascular disease, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders.

The alcoholic extract of roots and rhizomes of Podophyllum species (Berberidaceae) is used to treat the bites of venomous snakes and as purgative, anthelminthic, vesicant and suicide agents in America approximately 500 years ago.

The dried roots and stems of *Kadsura coccinea* (Schisandraceae), which contain at least ten dibenzocyclooctene-derived lignans, are included in the Chinese Pharmacopoeia for treatment of rheumatoid arthritis and gastric and duodenal ulcers.

Schisandra (Schisandraceae) fruits, which include lignans effective against chronic viral hepatitis and with protective action against liver damage by hepatotoxins, are used successfully to treat hepatitis. The dried bark of *Fraxinus mandshurica* (Oleaceae), which contains at least eight different lignans, and *Fraxinus japonica* are used as a diuretic, antipyretic, analgesic and antirheumatic in China and Japan, respectively. The bark of *Olea europaea*, from which a group of lignans has been isolated, was used in the Eastern world as an antipyretic, antirheumatic, and tonic agent, and the lignans are believed to be responsible for such properties.

Podophyllotoxin, a cyclolignan abundant in Podophyllum species, is used as an antiviral agent for the treatment of *Condiloma acuminata* and other types of warts including planar warts and genital warts and corns. Three derivatives of podophyllotoxin, etoposide, teniposide, and etopophos, have improved pharmacological profiles over the parent compound podophyllotoxin. These derivatives have been developed and are used clinically in treatment of different types of cancer.

Masoprocol, isolated from *Larrea tridentata*, is a useful topical agent for the prevention and treatment of pre-malignant skin lesion induced by actinic processes.

The flavolignans of *Silybum marianum* (Compositae) fruit are antihepatotoxic agents in therapeutic use.

Lignans have antioxidant, antibacterial, antiviral, fungicidal, insecticidal, estrogenic, antiestrogenic, anticarcinogenic, anticardiovascullar properties. The lignans consumed by animals and humans are hydrolyzed and metabolized by the intestinal microflora mainly to enterolignans, enterodiol, and enterolactone. These metabolites, known as mammalian lignans, possess a chemical structure similar to a sex hormone in the oestrogen family and can act as phytoestrogens.

Flaxseed, the richest source of mammalian lignan precursors, decreases some early markers of colon health risk.

Lignan-rich foods are high in fibre helps with digestion, cleans the colon, and removes toxins from the body. Lignans may decrease the risk of breast cancer in women and reduce the spread and growth of breast cancer after diagnosis.

Vegetarian men (with diets high in plant lignans) have better prostate health than omnivorous males, i.e. is an animal that has the ability to eat and survive on both plant and animal matter. Plant lignans give rise to the mammalian lignans enterodiol and enterolactone; the richest source is linseed (flaxseed).

Commercial applications: Lignan extract capsules are used as a dietary supplement. These capsules support cardiovascular health, promote cell health and act as an antioxidant.

Podophyllum resin powder is a topical medication used to treat skin warts including genital warts and plantar warts.

A homeopathic medicine containing Podophyllum resin, benzoin, aloes, ethanol, and isopropyl alcohol is used to treat warts. A Podophyllinum Mother Tincture is a homeopathic remedy. It helps to treating gastro-enteritis, swelling in liver, infections such as cholera and helps in reducing pain in uterus. It is effective against ulceration and piles of intestines.

Podophyllum is a homeopathic remedy made from the plant *Podophyllum peltatum* (May appleP. It is prescribed when the intestines, rectum and the liver are affected. It works against abdomen gastroenteritis with vomiting of bile and colic pain in the stomach, and jaundice with pain in the liver region better by rubbing part.

A formulation with Sesame lignans and Olive fruit extract 120 soft gels is used as a for longer life, promotes a healthy heart, and a dietary supplement.

Sesamin capsules contains sesame seed lignans and used as a natural dietary supplement. Each capsule contains sesame seed extract, sesamin, and sesamolin. These capsules have health promoting properties.

Tea

Biological source: Tea is an aromatic beverage prepared by pouring boiled water over the young cured or fresh leaves and leaf buds of the tea plant, *Camellia sinensis*, an evergreen shrub native to East Asia. Tea is also rarely prepared from the leaves of *Camellia taliensis*. Two principal varieties are used, the small-leaved China plant (*C. sinensis* variety *sinensis*) and the large-leaved Assam plant (*C. sinensis* variety *assamica*).

Family: Theaceae.

Tea plants are native to East Asia. Probably tea is originated near the Irrawaddy River in Myanmar (Burma) from where it spread out into southeast China, Indo-China and Assam. The natural home of the tea plant is considered in area between Nagaland, Manipur and Mizoram, Myanmar frontier in the west, through China in the Zhejiang Province, and from Thailand to Vietnam.

After plain water, tea is the most widely consumed drink in the world. It is the most popular beverage consumed by two-thirds of the world's population. There are many different types of tea; some have a cooling, slightly bitter, and astringent flavour, while others have sweet, nutty, floral, or grassy notes. Tea has a stimulating effect in humans, primarily due to its caffein content.

A tea plant is an evergreen shrub that may grow into a tree up to 16 m in height if left undisturbed. The optimal temperature range is from 15 to 20 °C. The cultivated plants are small for ease of plucking. Only the top 2.5–5 centimetres of the mature plant are picked. A plant will grow a new flush every 7 to 15 days during the growing season. Leaves that are slow in development tend to produce better-flavoured teas.


Tea leaves

Classification of teas: Teas are classified according to region of origin, as in China, Ceylon, Japanese, Indonesian, and African tea, or by smaller district, as in Darjeeling, Assam, and Nilgiris from India, Uva and Dimbula from Sri Lanka, Keemun from Chi-men in China's Anhwei Province, and Enshu from Japan.

Tea types are also based on processing or harvested leaf development, e.g. black tea (fermented), green tea (non-fermented), oolong tea (semi-fermented), and white tea (least processing). These major tea types differ in how tea is produced and processed according to the different processes of drying and fermentation that determine its chemical composition.

In modern commercial grading, 95 to 100 percent of production belongs to broken grades. There is an increased demand for teas of smaller particle size, which produce a quick, strong brew. Green tea is usually produced from the China plant and is grown mostly in Japan, China, and to some extent Malaysia, India and Indonesia.

Processing the leaf: In tea manufacture, the leaf goes through the stages of withering, rolling, fermentation, and drying. During this process, the leaves are dried and the chemical constituents of the leaves are allowed to produce the quality peculiar to each type of tea. Only black tea goes through all stages of the manufacturing process. Green tea and oolong tea acquire their qualities through variations in the crucial fermentation stage.

Withering: Plucking the leaf initiates the withering stage, in which the leaf becomes flaccid and loses water until, from a fresh moisture content of 70 to 80 percent by weight, it arrives at a withered content of 55 to 70 percent, depending upon the type of processing.

In the traditional process, fresh leaf is spread by hand in thin-layers onto trays or sections of coarse fabric called tats. It is then allowed to wither for 18 to 20 hours, depending upon several factors that include the temperature and humidity of the air and the size and moisture content of the leaf.

Rolling: At this stage, the withered leaf is distorted, acquiring the distinctive twist of the finished tea leaf, and leaf cells are burst, resulting in the mixing of enzymes with polyphenols. The traditional method is to roll bunches of leaves between the hands, or by hand on a table, until the leaf is twisted, evenly coated with juices, and finally broken into pieces.

Fermentation: Fermentation commences when leaf cells are broken during rolling and continues when the rolled leaf is spread on tables or perforated aluminum trays under controlled conditions of temperature, humidity, and aeration. The process actually is not fermentation at all but a series of chemical reactions. The most important is the oxidation by polyphenol oxidase of some polyphenols into compounds that combine

with other polyphenols to form orange-red compounds called theaflavins. The theaflavins react with more units to form the thearubigins, which are responsible for the transformation of the leaf to a dark brown or coppery colour. The thearubigins also react with amino acids and sugars to form flavour compounds that may be partly lost if fermentation is prolonged. In general, theaflavin is associated with the brightness and brisk taste of brewed tea, while thearubigin is associated with strength and colour.

Drying: At this stage, heat inactivates the polyphenol enzymes and dries the leaf to a moisture content of about 3 percent. It also caramelizes slowly to turn sugars to add flavours to the finished product, and imparts the black colour associated with fermented tea. The fermented leaves are dried by heated forced air. A mechanized drier consists of a large chamber into the bottom of which hot air is blown as the leaf is fed from the top on a series of descending conveyors. The dried leaf is then cooled quickly to prevent over drying and loss of quality.

Black tea: It is made by first exposing the tea leaves to air for oxidation. This oxidation process turns leaves into a deep brown color and during this process, the flavor is intensified. The leaves are then left as such or are heated, dried and crushed.

Green tea: For preparing unfermented tea, the oxidizing enzymes are killed by steam blasting the freshly plucked leaf in perforated drums or by roasting it in hot iron pans prior to rolling. The leaf is then subjected to further heating and rolling until it turns dark green and takes a bluish tint. The leaves are finally dried to a moisture content of 3 to 4 percent and are either crushed into small pieces or ground to a powder.

With the inactivation of polyphenol oxidase, the polyphenols are not oxidized and therefore remain colourless, allowing the processed leaf to remain green. The absence of theaflavins and thearubigins in the finished leaf also gives the beverage a weaker flavour than black tea.

Matcha tea: Matcha, a form of green tea, must be taken from shade-grown tea plants, which have heightened levels of chlorophyll and have a bright green colour, and only the buds and top three layers of the young tea plant (*Camellia sinensis*) are harvested. The tea leaves are steamed to halt the oxidation process, then deveined and ground in stone mills. Because of such laborious and exacting production standards, matcha is among the most expensive kinds of teas in the market.

Matcha is a very fine, high-quality green tea powder made from the entire leaves of tea bushes grown in the shade. Since it is the only form of tea in which the leaves are ingested, matcha tea contains even more antioxidants that regular green tea. In fact, some have suggested that one cup of matcha tea is the equivalent to 10 cups of regular green tea.

Oolong tea: Oolong tea is produced by a partial oxidation of the leaf, intermediate between the process for green tea and black tea. After a brief withering stage, the leaf is lightly rolled by hand until it becomes red and fragrant. For oolong tea it is then fermented for about one-half, and for pouching tea (a lightly oxidized tea) for one-quarter, of the time allowed for black tea. Fermentation is stopped by heating in iron pans, and the leaf is subjected to more rolling and heating until it is dried.

Packaging: Sorting and grading are the first steps in packaging tea by particle size, shape, and cleanliness. This is carried out on mechanical sieves or sifters fitted with meshes of appropriate size. With small-sized teas in demand, some processed teas are broken or cut again at this stage to get a higher proportion of broken grades.

Undesirable particles, such as pieces of tough stalk and fibre, are removed by hand or by mechanical extractor. Winnowing by air removes dust, and fibres.

Teas are packed in airtight containers in order to prevent absorption of moisture, which is the principal cause of loss of flavour during storage. Packing chests are usually constructed of plywood, lined with aluminum foil and paper, and sealed with the same material. Also used are corrugated cardboard boxes lined with aluminum foil and paper or paper sacks lined with plastic.

Blended teas are sold to consumers as loose dust tea, which is packed in corrugated paper cartons lined with aluminum foil, in metal tins, and in fancy packs such as metallized plastic sachets, or they are sold in tea bags made of special porous paper. Tea bags are mainly packed with broken-grade teas.

Teas are packaged in tea bags, tea sachets, or as loose-leaf. Loose-leaf teas sold in tin canisters or sacks allow you to control how much tea to use, using more to create a stronger flavor or less for more mellowness. Tea bags and sachets hold a standard amount of leaves for optimum flavor and are portable.

Black and oolong teas are generally steeped in hot or boiling water (about 98.88 °C) and brewed for about 4–5 minutes. Green tea is steeped at a slightly lower temperature 82.22 °C from 4–15 minutes. The longer tea steeps, the stronger the flavor with bitter notes.

Additives of sugar, cream, or milk can reduce the polyphenol content of tea. For the greatest health benefits, try serving tea plain or without too many additives. A dash of vanilla or cinnamon can mimic sweetness. Some fruit-flavored herbal teas taste naturally sweet to the palate without added sweeteners.

A tea infusion is best made by pouring water just brought to the boil over dry tea in a warm teapot and steeping it from three to five minutes. The liquor is separated from the spent leaves and may be flavoured with milk, sugar, or lemon.

Chemical constituents: Green tea contains polyphenolic compounds, caffeine (3.5%), theobromine, theophylline, lignin (6.5%), (–)-epicatechin, (–)-epigallocatechin-3-gallate, (–)-epigallocatechin, and (–)-epicatechin-3-gallate. Flavonols, including quercetin, kaempferol, myricetin and their glycosides are also present in tea.

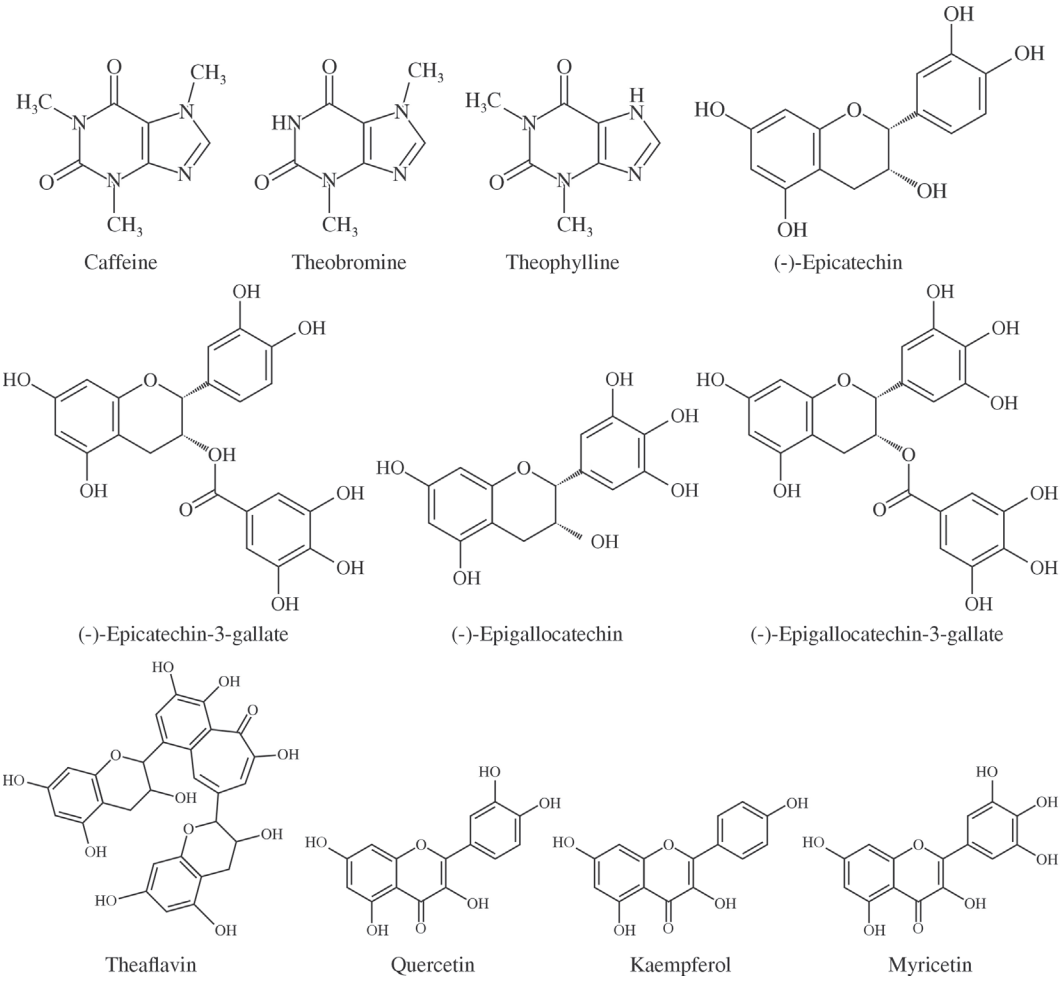
A typical cup of green tea usually contains 250–350 mg tea solids, of which 30–42% are catechins and 3–6% caffeine. It also contains organic acids, chlorophyll, thiamine (4.0%), and free amino acids.

The major active constituents of tea are catechins. Some catechins are oxidized or condensed to theaflavins (theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3–3′-digallate) (3–6%) and thearubigins (12–18%) during fermentation of fresh tea leaves and are responsible for the bitter taste and dark color of black tea. Black tea contains mainly thearubigins, theaflavins, flavonols and catechins. The most favourable effects of tea are accredited to the polyphenols and caffeine.

The best-known constituent of tea is caffeine, which gives the beverage its stimulating character but contributes only a little to colour, flavour, and aroma. About 4 percent of the solids in fresh leaf is caffeine, and one teacup of the beverage contains 60 to 90 milligrams of caffeine. Tea also contains small amounts of theobromine and theophylline, which are xanthine and stimulants, similar to caffeine.

The astringency in tea is attributed to the presence of polyphenols. These are the most abundant compounds in tea leaves, making up 30–40% of their composition.

Polyphenols in tea include flavonoids, epigallocatechine gallate, and other catechins. The taste and the flavour of tea are enhanced due to the presence of polyphenols, caffeine, organic acids and volatile terpenes. The characteristic taste of green tea is a mixture of bitterness, umami taste, sweetness and slight sourness. The aroma of tea is enhanced by volatile organic compounds such as terpenoids, alcohol and carbonyl compounds.



Storage: Tea must be kept at room temperature in an air-tight container. Black tea in a bag within a sealed opaque canister may keep for two years. Green tea deteriorates more rapidly, usually in less than a year. Tightly rolled gunpowder tea leaves keep longer than the more open-leafed teas. Storage life for all teas can be extended by using desiccant or oxygen-absorbing packets, vacuum sealing, or refrigeration in air-tight containers.

There are five elements to avoid to keep tea as fresh as possible, e.g. light, heat, moisture, odor, and air. Tea bags should be stored in their original container or placed in a sealed plastic bin. Loose-leaf teas should be stored in an airtight container. Place all teas in a dark cupboard at a consistent room temperature. Tea tends to absorb odors from food and even other strongly scented teas, so keep them separate. Freezing and refrigerating is not recommended as the moisture introduced can degrade the tea.

If unopened, tea will last about one year beyond the date. After opening, packaged and loose-leaf teas last about one year. However, some black and oolong teas can last up to two years, and more delicate teas may last only 6 months. The flavor is the best guide to determining how long to keep a tea in a cupboard.

Extraction of caffeine: Caffeine dissolves in hot water. The tannins and gallic acid are also soluble in water. Calcium carbonate converts these tannins and gallic acid into insoluble salts. Caffeine is a minor constituent of tea, coffee, and other natural plant materials. The major constituent of tea is cellulose which is not water soluble.

Procedure: Add about 10.0 g of tea leaves to 100 ml of water in a 400 ml beaker. Add 5 g of calcium carbonate and boil this suspension for about 10 minutes. Add 3 g of filter acid (celite) and decant the mixture into a Buchner funnel. Any unfiltered solid on the filter paper can be discarded. The caffeine is present in the filtrate.

Cool the filtrate to room temperature and pour it into a 125 ml separatory funnel. Add 10 ml of methylene chloride to the funnel and shake gently. Gases will be produced, so it is important to vent the mixture periodically by taking the cap off. Caffeine can then be extracted from the water by nonpolar methylene chloride in almost pure form. Some chlorophyll is often extracted at the same time. To break the emulsion formed in the methylene chloride layer, slowly drain the methylene chloride layer through a small amount of anhydrous magnesium sulphate in a powder funnel with a loose cotton plug.

Add 10 ml of fresh methylene chloride to the funnel and once again shake gently with venting. This is just giving another chance for any caffeine left behind in the water layer to dissolve in the organic layer. Open the stopcock to allow the organic layer out, and combine it with the other organic layer.

The methylene chloride extract is evaporated on a steam bath to obtain caffeine. The final caffeine should be white. Occasionally, it has a greenish tinge, which is fine. Any yellow or brown color is a sign of impurities.

Analysis of caffeine: Caffeine (1,3,7-trimethylxanthine, $C_8H_{10}N_4O_2$), molecular weight 194, occurs as a bitter white crystalline alkaloid, melting point 235–237 °C. It is a common ingredient in a variety of drinks (soft and energy drinks) and is also used in combination with various medicines. In order to maintain the optimum level of caffeine, various spectrophotometric methods have been developed. The monitoring of caffeine is a very important aspect because of its consumption in higher doses that can lead to various physiological disorders.

Caffeine is extracted from the product using dichloromethane. The extract is analyzed using thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS) to identify caffeine and other trace components.

The developed TLC method is successfully applied for the determination of caffeine in tea samples. The method is easy to handle and also can be used in the routine analysis for the determination of caffeine content in samples of green, black, and white teas.

Analysis procedure: The isolated sample is dissolved either in 1 ml methanol. The Merck precoated silica gel plate 5×10 cm is used for the TLC analysis and then the plates were visualized at 254 nm using UV lamp. A mobile phase solvent system consisting ethyl acetate and ethanol (8:2, v/v) is used to develop the TLC plate. The R_f value from the analysis of pure caffeine is 0.71. The R_f values indicate the results of the qualitative analysis of pure caffeine.

Therapeutic uses: Tea improves blood pressure, gut health, and cardiovascular health, reduces diabetes and cancer risks, alleviates stress and anxiety, and supports bone health.

Caffeine is a stimulant present in tea, coffee, cola beverages, analgesic drugs, and agents used to increase alertness. It is also used to prevent and treat pulmonary complications of premature birth. Caffeine increases focus and memory, improves alertness, concentration, person's athletic performance, and alleviate both migraines and tension headaches. Caffeine is also present in a large number of products which are used as the counter pain relievers, headache remedies, and antihistamines.

Caffeine can cause adverse effects, especially if people consume it in large quantities, or if they have a caffeine sensitivity. These side effects include anxiety, nervousness, diarrhoea, dizziness, dysphoria, fast heart rate, headache, heartburn, high blood pressure, nausea, shaking, sleep problems, and thirst.

Herbal teas contain a blend of herbs, spices, fruits or other plants in addition to tea leaves. Herbal teas do not contain caffeine. These teas include chamomile, peppermint, vanilla, turmeric, ginger, and fruit essence teas. They may have notes of floral, fruit, mint, spice, grassiness, sweetness, or bitterness. The varieties are vast, and the choice is completely up to personal preferences. There are numerous types of herbal teas. Some of the most popular herbal teas include:

Chamomile tea: It helps to reduce menstrual pain and muscle spasms, improves sleep and relaxation, and reduces stress.

Rooibos: It improves blood pressure and circulation, boosts good cholesterol while lowering bad cholesterol, keeps hair strong and skin healthy, and provides relief from allergies.

Peppermint tea: It contains menthol, which can soothe an upset stomach and serve as a cure for constipation, irritable bowel syndrome and motion sickness. This tea variety also relives pain from tension, headaches and migraine.

Ginger tea: It helps to fight against morning sickness, can be used to treat chronic indigestion and helps to relieve joint pain caused by osteoarthritis.

Hibiscus tea: It lowers blood pressure and fat levels, improves liver health, can starve off cravings for unhealthy sweets, and may prevent the formation of kidney stones.

Oolong tea is notable for containing l-theanine, an amino acid that reduces anxiety and increases alertness and attention.

Tea polyphenols kill the cancer cells. The use of green tea-supplemented food products promotes health benefits. It prevents the cancer and these products can be included as a dietary supplement for cancer fighters.

Commercial applications: Tea is the most popular manufactured drink consumed in the world among coffee, soft drinks, and alcohol. India is the world's largest tea-drinking nation.

Green tea has gained popularity among people as a functional food having both nutritional and beneficial therapeutic effects leading to the growth of the global green tea market.

The demand for catechin based health supplements and organic preservatives are on the rise among health-conscious consumers and food processing companies. Commercially available green tea in a grained powder form and tea extract can be solubilized in water.

Green tea extract is used for many food products including bread, biscuits, dehydrated apple and meat products. In a food industry, the major problem is lipid oxidation and it induces the potential toxicity in food products. The main application of green tea extract is spraying in many food products and it showed comparable antioxidant performance to conventional synthetic antioxidant tert-butylhydroquinone (TBHQ). Furthermore, green tea extract is more cost-effective than other natural sources.

Tea catechin has high potential for the inhibition of lipid peroxidation in foods. The green tea catechins are highly efficient to prevent the lipid oxidation when supplemented with meat and meat products. The catechins are used to prevent the lipid oxidation in meats by chelating iron, which is the major active catalyst for oxidative rancidity in meat. Furthermore, tea catechins trap the peroxy radicals and suppress the free radical chain reactions; finally, it prevents the lipid oxidation in meat products. The beneficial effect of catechins minced with meat and meat products improved their quality and enhanced shelf-life with additional antioxidant potentials to consumers. Furthermore, catechins in meat and meat products are rich in iron, because catechins can bind with iron to reduce its absorption in the body.

Tea catechins have wide applications in fish and fish products in order to enhance the shelf-life and health benefits.

The green tea catechins are supplemented with plant food products to extend the shelf-life and health benefits. For example, catechins are added in vegetables, oils, cakes, starch, bread and juice to extend their shelf-life and health benefits of their products.

Tea can be used in make-up products such as eyeliner, mascara, eyeshadow, blush and foundations. It is also used in fragrances, hair care products, hair dyes, wave sets, shaving products, sunscreens and skin care and skin cleansing products.

Caffeine injection, known as Cafcit, is used intravenously. Caffeine solution is used orally. Caffeine and Sodium Benzoate is administered intravenously. Caffeine citrate injection is used intravenously. Caffeine citrate solution is taken orally.

Alert (tablet), Alert Aid (tablet), and Alert Aid caffeine Capsules (175 mg) are prescribed orally. Tablets containing Caffeine (15 mg) + Acetaminophen (500 mg) + Codeine phosphate (8 mg), are used orally. Tablets composed of Caffeine citrate (30 mg/tab) + Acetylsalicylic acid (325 mg/tab) are administered orally. Tablets composed of Caffeine citrate (30 mg) + Acetyl salicylic acid (375 mg) + Codeine phosphate (8 mg) are used orally. Tablets containing Caffeine citrate (30 mg) + Acetylsalicylic acid (350 mg) + Codeine phosphate (15 mg) + Meprobamate (200 mg) are utilized orally.

Ruta

Biological source: Ruta consisted the whole herb *Ruta graveolens* L.

Family: Rutaceae

Its common names are Garden rue, Herb of grace, Rue, Rue-bitterwort, Rue oil, Ruta-de-smell strong, Rue-smelly, Sadab, and Satab.

It is a native to the Balkan Peninsula, Mediterranean region of southern Europe and northern Africa. It is cultivated as a medicinal and ornamental herb as a low hedge in many countries including India, especially for its bluish leaves, as a culinary herb, and as an insect repellent and incense.

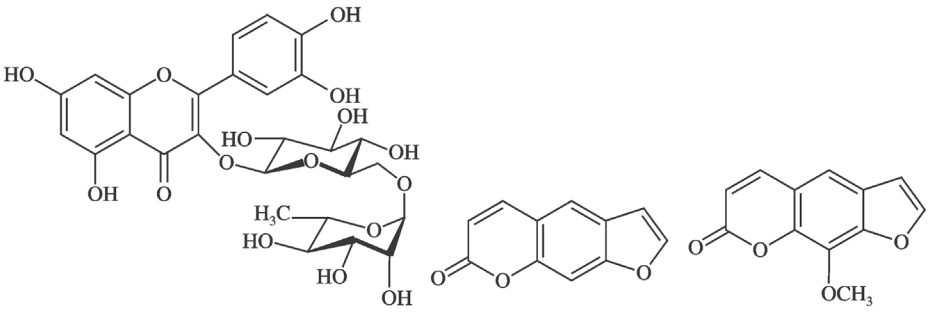
Ruta graveolens L. is a woody, perennial, evergreen, aromatic, and glabrous herb or a sub-shrub. Stem is slender, smooth, pale glaucous green and reaches up to a meter in height.

Leaves are fern-like, oblong, blue green, alternate, gland-dotted, glaucous, compound, 2–3 pinnate. Flowers are small, regular bisexual, terminal ones are pentamerous and others are tetramerous. Petals are distinct, in clusters, widely spreading, greenish yellow, wide and hooded at top, margin wavy and sometimes toothed. Fruits are dry, hard, and roundish. It grows well in full sun to part shade, moderately fertile, moist, well-drained soil.

Chemical constituents: *Ruta graveolens* leaves contain flavonoids rutin and quercetin, linear furanocoumarins, psoralen and methoxypsoralen, and an alkaloid named graveoline.

The roots yielded acridone alkaloids rutacridone, rutacridone epoxide and gravacridondiol.

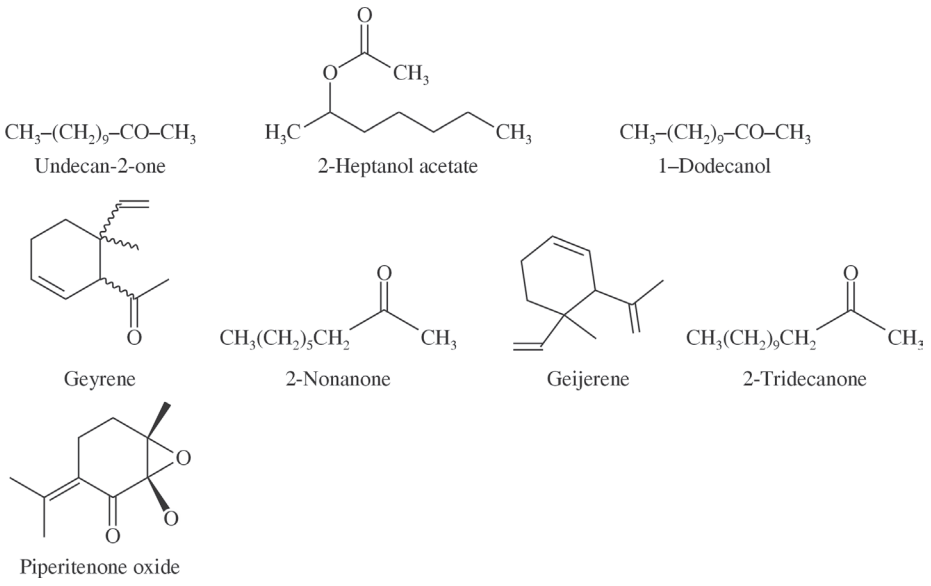
Ruta graveolens contains acridone alkaloids such as arborine, evoxanthine, furacridone and gravacridone, quinoline alkaloids, for example, graveoline and graveolinine, dictamnine, coumarins like gravelliferone, isorutarin, rutacultin, rutaretin, and suberenone, the furanocoumarins 5-methoxypsoralen (bergapten), chalepensis, and 8-methoxypsoralen (xanthotoxine); carotenoids, chlorophyll and limonoids.



The flavouring aerial parts contain a volatile oil composed of aliphatic acids, alcohols and ketones including undecan-2-one (33.9–47.21%), 2-heptanol acetate (17.5%), 1-dodecanol (11.0%), geyrene (10.4%), 2-nonanone (18.8–39.17%), geijerene, 2-tridecanone, 2-decanone, trans-piperitenone oxide, cis-piperitenone oxide, elemol, 2-methyl-undecanal, 2-dodecanone, 2-nonanol, octyl acetate, diethyl phthalate, and pentadecanolide acetate.

The major compounds of aerial part volatile oil from Jordon were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%), and 2-decanone (3.31%).

Other compounds in the essential oil included 2-acetoxy tetradecane (14.49%), aliphatic ester nonyl cyclopropanecarboxylate (9.22%), 1-methyl-5, 6-divinyl-1-cyclohexene, and 2-undecanol.



Extraction and isolation of the essential oil: The aerial parts of the plant (500 g) were hydrodistilled in a Clevenger type glass apparatus for 4 hour. The volume of the oil was collected in the graduated tube. The collected pale yellow essential oil was dried over anhydrous sodium sulphate and stored at 4 °C in the dark. The yield was of the essential oil was 2.8% based on dry weight of the sample. This oil was used for GC and GC-MS analysis.

Gas chromatographic (GC) analysis: The gas chromatographic analysis of the essential oil was carried out on a GC-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a flame ionization detector (FID) and ULBON HR-1 fused silica capillary column (60 m × 0.25 mm × 0.25 µm). The injector and detector (FID) temperatures were maintained at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230 °C at a rate of 3 °C/min and then held at 230 °C for 9 minutes, with a total run time of 55.14 minutes. Percentage of the constituents were calculated by electronic integration of FID peak areas. Injection volume for all samples was 0.1 pi.

Gas chromatographic–mass spectroscopy (GC-MS) analysis: The GC-MS analysis was carried out on a GC-MS-QP 2010 Plus (Shimadzu Scientific Instruments, Kyoto, Japan) fitted with a Column AB-Innowax (60 m × 0.25 mm i. d., film thickness 0.25 µm). The carrier gas was nitrogen at a flow rate 1.21 ml/minutes. The oven column temperature was initially kept at 60 °C for 10 min and increased up to 230 °C at a rate of 4 °C/minutes, then held at 230 °C for 10 min and increased up to 260 °C at a rate of 1 °C/min and then held at 260 °C for 10 minutes. The split flow was 10 ml/minutes. The injector temperature was 240 °C and detector temperature was 280 °C. Injection volume was 0.3 µl. The ionization energy (voltage) was 70 eV and mass scan range (m/z) was 40–850 amu. The percentage composition of the oil was calculated automatically from the FID peak area without any correction.

Identification of essential oil: The individual compounds were identified by comparing their Kovat's indices (KI) of the peaks on Innowax fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was carried out by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature. Relative amounts of identical components were based on peak areas obtained without FID response factor correction. The retention indices were calculated for all compounds using a homologous series of n-alkanes under the same operational conditions of analysis.

The essential oil components were identified by comparing their retention indices of GC chromatograph with those of literature. Further identification was done by GC-MS. The fragmentation patterns of mass spectra were compared with those of the spectrometer data base using the NBS 54 AL and Wiley L-built libraries and with those reported in the literature. Many constituents were identified by comparing their retention indices with those of authentic standards available in the author's laboratory.

Isolation of rutin: The dried aerial parts powder (200 g) of *Ruta graveolens* was extracted by Soxhlet apparatus with 80% ethanol till exhaustion. The alcoholic extract was filtered and concentrated by evaporation under vacuum to about 100 ml. It was then mixed with 250 ml distilled water, and extracted with petroleum ether (500 ml × 3), then with chloroform (500 ml × 3).

After extraction, the aqueous layer was collected and left to stand in a cold place for 72 hours. A yellow precipitate separated out of the solution. The precipitate was filtered and washed with a mixture of chloroform: ethyl acetate: ethanol (50:25:25). The un-dissolved part of the precipitate was dissolved in hot methanol and filtered. The filtrate was evaporated to dryness to give a yellow powder (rutin). Its melting point 242 °C was measured.

Identification of isolated rutin: Identification of rutin was performed by TLC using mixtures of solvents and UV light. TLC test served not only to identify rutin through R_f values, but also to determine the purity of the extracted substance. The solvents system for development of the TLC plate is ethyl acetate-acetic acid 1:1, v/v (R_f 0.44).

Isolated rutin was also compared with standard rutin using TLC method; a pre-coated aluminum sheet with silica gel GF254 with the mobile phases; e.g. (a) ethyl acetate: butanone: formic acid: water (50:30:10:10), and (b) ethyl acetate: formic acid: acetic acid: water (100: 11: 11:27). In paper chromatography, Watman No.1 filter paper was used as a stationary phase and mobile phases such as (a) acetic acid: water (15:85) and (b) isopropyl alcohol: water (60:40).

The isolated rutin was dissolved in methanol and its UV absorption peaks were determined and compared with standard rutin. Infrared spectrum of the isolated rutin was determined using KBr disk method.

Therapeutic uses: *Ruta graveolens* is used to treat eye problems, arthritis, pain, rheumatism, dermatitis, inflammatory diseases, dislocations, tendon strains, varicose veins, intestinal disorders and skin conditions such as psoriasis and eczema.

The whole herb is abortifacient, anthelmintic, antidote for venom, antispasmodic, carminative, emetic, emmenagogue, expectorant, haemostatic, insect repellent, ophthalmic, rubefacient, strongly stimulant, mildly stomachic and uterotonic. An infusion of the herb is used to treat hysterical affections, coughs, and flatulence. The

juice of the plant is dropped to relieve earaches. Chewing a leaf or two relieves from giddiness, nervous headaches, palpitations.

A homeopathic tincture obtained from the fresh herb before flowering begins is used to cure eye strain, headache and sprains.

The growing or the dried plant can be used to repel insects. It is most useful when the plant is grown near roses and raspberries. The dried herb can also be put in the linen cupboard to repel moths. An essential oil obtained from the leaves and young shoots is used in perfumery and as a food flavouring.

If *R. graveolens* is externally spread on moist skin under direct sunlight, it leads to photosensitivity, caused by furanocoumarins resulting in hyperpigmentation, swelling, itching, and even burns and blistering. Ruta extracts are mutagenic and hepatotoxic. Its large doses can cause violent gastric pain, vomiting, liver damage, and death. It is recommended to only use small amounts in food.

It is not recommended for use by pregnant or lactating women because in high concentrations it can provoke hyperemia in the uterus and high mobility (oxytocic action) which may cause abortion and is teratogenic. Hyperemia occurs when excess blood is produced inside the vascular system, which is the system of blood vessels in the body.

R. graveolens is rich in rutin which acts as a venotonic drug affecting the veins and capillary protector. Rutin helps to increase visual sharpness. It is useful against edema, inflammation, thrombogenesis, spasms, and hypertension.

The Ruta essential oil is antispasmodic, anti-epileptic, rubefacient, spasmolytic, anti-inflammatory, antihistaminic, emmenagogue, and vermifuge. It has a depressing effect on the central nervous system.

At high doses, Ruta essential oil works as a narcotic poison, provoking violent intestinal inflammation, tongue and larynx tumefaction, excitement, followed by fatigue, vertigo, mental confusion, trembling, nephritis with uterine swelling and abortion, liver damage, intestine damage, and eventually death. The essential oil can cause contact dermatitis and phototoxic reactions and severe hepatic and renal toxicity.

The plant is used to season some food items such as soup, cheese, butter, coffee, and tea, and in medicinal preparations. Ruta is combined with the poisonous shrub oleander and drunk as an antidote to venomous snake bites. Most cats dislike the smell of it. Therefore, it can be used as a deterrent to them.

Ruta has culinary uses. But it is bitter and causes gastric discomfort in some individuals. Due to small amounts of toxins present in Ruta, it must be used in small amounts, and should be avoided by pregnant women and by those women who have liver issues.

Therapeutic doses of Ruta can lead to depression, sleep disorders, fatigue, dizziness, and cramps. The sap from fresh leaves can produce painful gastrointestinal irritation, fainting, sleepiness, a weak pulse, abortion, a swollen tongue, and cool skin.

Commercial applications: Rutin tablets are manufactured by Lupin Ltd., commonly used for the diagnosis or treatment of varicose veins, diabetic retinopathy, hemorrhoids, and chronic venous insufficiency. The tablets have some side effects such as central nervous system depression, coma, ectopic mineralization, and gastrointestinal disorder.

A homeopathic drug of *Ruta graveolens* with dilutions 6C, 30C, 200C, 1M, and 10M is an effective remedy to treat eyes, uterus and cartilages diseases, e.g. arthritis and rheumatic pains. It relieves pain in the muscle joints, tendons, heals bruises and facilitates quick recovery from an injury or a fracture. It also provides quick relief from soreness in the ankles and lower back.