COUMARINS

Among them, coumarins are a family of benzopyrones (1,2-benzopyrones or 2H-1-benzopyran-2-ones) widely distributed in the nature. They represent an important family of naturally occurring and/or synthetic oxygen-containing heterocycles, bearing a typical benzopyrone framework (Figure 1) [1].

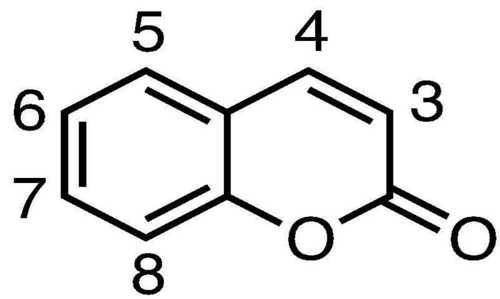

Figure 1.

Chemical structure of coumarin and the IUPAC numeration of this scaffold.

The name coumarin comes from a French term for the Tonka bean, *coumarou*, seeds of *Dipteryx odorata* (*Coumarouna odorata*) (*Fabaceae*/*Leguminosae*), one of the sources from which coumarin was first isolated as a natural product in 1820. It has a sweet odor, easy to be recognized as the scent of new-mown hay; because of that, coumarin has been used in perfumes since 1882. It is presumed to be produced by plants as a chemical defense to discourage predation [2, 3].

Coumarin compounds are a class of lactones structurally constructed by a benzene ring fused to α-pyrone ring, and essentially possess - conjugated system with rich electron and good charge-transport properties [4, 5]. The simplicity and versatility of the coumarin scaffold make it an interesting starting-point for a wide range of applications [6-8]. There are coumarins as perfumes, cosmetics, and industrial additives. Some of its derivatives have been used as aroma enhancers in tobaccos and certain alcoholic drinks [9, 10]. But their most relevant role is described in natural products, organic chemistry, and medicinal chemistry [11, 12]. The extraction, synthesis, and evaluation of coumarins have become an extremely attractive and rapidly developing topic [13, 14]. Moreover, a lot of coumarin compounds as medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, better curative effects, etc., to treat various types of diseases are being actively studied [15]. Several efforts have been made mainly in developing coumarin-based anticoagulant, antioxidant [16], antimicrobial (anti-viral, antifungal, and anti-parasitic) [10, 17], anticancer [18-20], anti-diabetic, analgesic, anti-neurodegenerative, and anti-inflammatory agents [10, 21]. Moreover, the unique and versatile oxygen-containing heterocyclic structure makes coumarin compounds occupy an important place in medicinal chemistry [22, 23]. In addition, studies have been done regarding coumarins as bioactive agents [24], as well as supramolecular medicinal drugs, diagnostic agents and pathologic probes, and biological stains [25]. Particularly, the large - conjugated system in the coumarinic ring, with electron-rich and charge-transport properties, is important in the interaction of this scaffold with molecules and ions. Coumarin-based ion receptors, fluorescent probes, and biological stains are growing quickly and have extensive applications to monitor timely enzyme activity, complex biological events, as well as accurate pharmacological and pharmacokinetic properties in living cells [26, 27].

Coumarin was first synthesized in 1868, and it was used in the pharmaceutical industry as a precursor in the synthesis of a number of synthetic anticoagulant pharmaceuticals, starting with dicoumarol (removed from the current therapy) [28]. So far, some interesting coumarin-based anticoagulant drugs have extensively been used in clinics [29]. Coumarins are a type of vitamin K antagonists [30]. The most notable ones are warfarin, acenocumarol, and phenprocoumon, currently in use in several countries [31, 32]. Warfarin is employed more frequently than acenocoumarol because of its longer half-life (36 h), theoretically providing more stable anticoagulation and avoiding factor VII fluctuations that potentially occur during acenocoumarol treatment (half-life 10 h) [33]. Nowadays, some coumarins proved to be enzymatic inhibitory agents [monoamine oxidase (MAO) inhibitors, acetylcholinesterase (AChE) inhibitors, and butyrylcholinesterase (BuChE) inhibitors] with great potential in neurodegenerative diseases (ND) [34-38]. These studies represent an important tendency in the coumarin's chemistry and biological evaluation [39-41].

Therefore, the coumarin ring is prevalently applied to construct several functional molecules in the medicinal field. A great deal of work has been done directed towards the separation and purification of naturally occurring biological coumarins from a variety of plants, animals, and microorganisms, as well as towards the artificial synthesis of coumarin compounds with novel structures and properties [42]. Coumarin compounds as medicinal drugs have been increasingly attracting special interest due to their underlying outstanding contributions in the prevention and treatment of diseases, and the related researches and developments have become an extremely attractive highlighted area.

In this context, an overview of the role of coumarins as important phytochemicals and their interesting applications will be presented and discussed. The origin, natural sources, biosynthesis, and applications are going to be presented in this chapter.

2. Naturally occurring coumarins

Coumarin (Figure 1) and its derivatives are an important group of natural compounds widely distributed in the natural kingdom [43]. They can be found in the integument of seeds, fruits, flowers, roots, leaves, and stems, although the largest concentration is generally in fruits and flowers [44]. Originally, coumarin was isolated from the seed of *D. odorata*. Coumarins are secondary metabolites of higher plants, few microorganisms (bacteria and fungi), and sponges [45]. The function of this type of end product of secondary metabolism is related to defense mechanisms against herbivores and attacks by microorganisms. These compounds are biosynthesized from phenylalanine via the shikimic acid [46]. Natural coumarins are generally unsaturated lactones and comprise another class of compounds C_6C_3. Almost all the natural coumarins have an oxygenated substituent at position 7 [47], either free as in hydroxylated umbelliferone, or combined (methyl, sugars, etc.) in other derivatives. Structurally, they are considered derivatives of the *ortho*-hydroxy-cinnamic acid.

There are different classifications for the coumarin derivatives. Generally, they can be chemically classified according to the most common cores: simple coumarins, complex coumarins, and various coumarins. More complex coumarins are generally fused with other heterocycles [3]. Therefore, they can be classified as: simple coumarins, furocoumarins, dihydrofurocoumarins, pyranocoumarins (linear and angular), phenylcoumarins, and biscoumarins [1]. As said before, hundreds of coumarins have been identified in natural sources, especially plants [48, 49]. Major coumarin constituents isolated from plants include: simple hydroxycoumarins, furocoumarins and isofurocoumarins, pyranocoumarins, biscoumarins, and dihydroisocoumarins (Figure 2) [1].

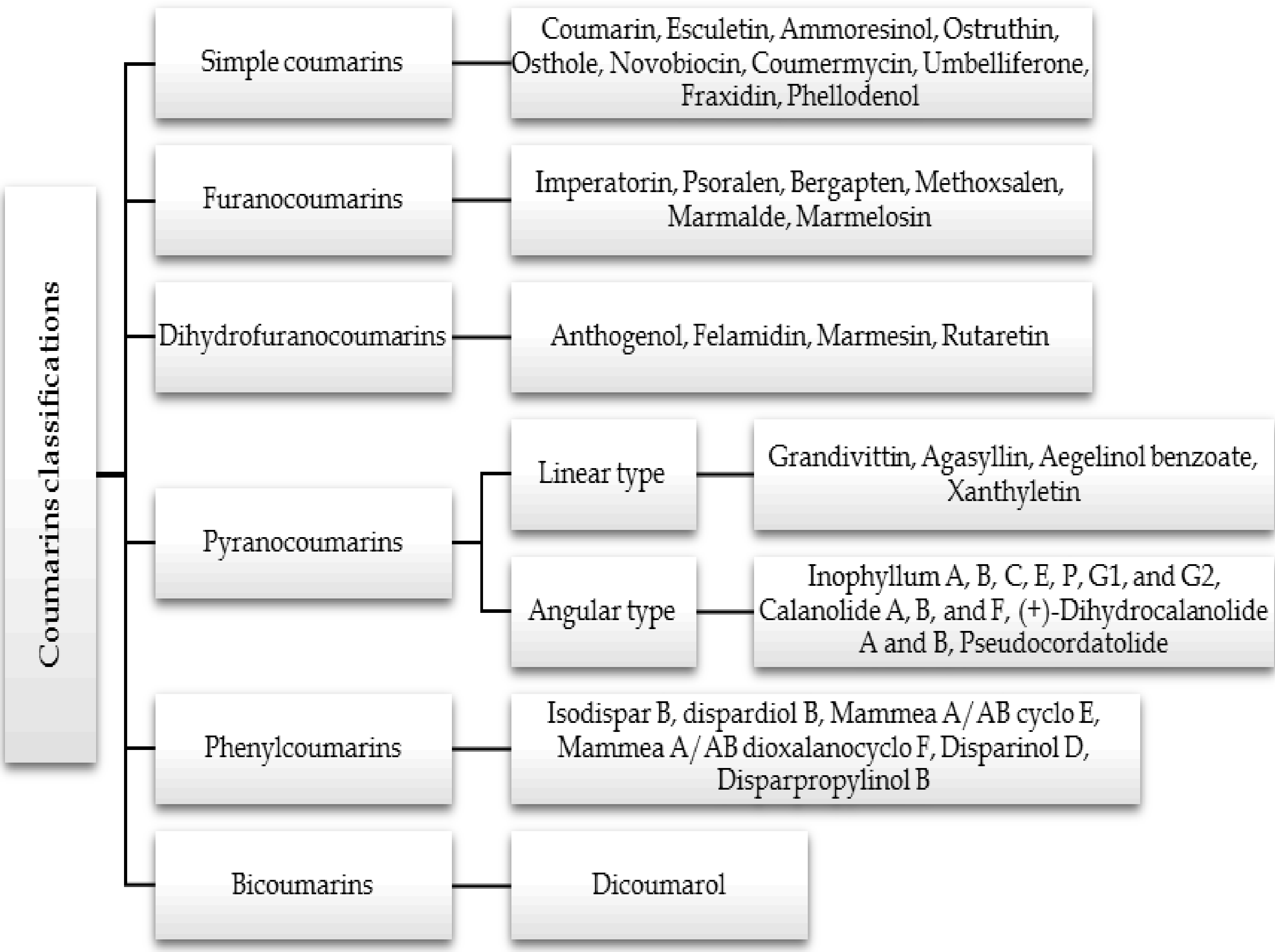

Figure 2.

Principal types of coumarins isolated from plants.

Coumarins have been isolated from hundreds of plants species distributed in more than 40 different families. There were isolated more than different 1300 coumarins, well distributed in *Angiospermae*, *Monocotyledoneae* and *Dicotyledoneae* families. Orders with occurrence numbers > 100 are *Araliales*, *Rutales*, *Asterales*, *Fabales*, *Oleales*, *Urticales*, and *Thymelaeales*. Families with occurrence numbers > 100 are *Apiaceae* (*Umbelliferae*), *Rutaceae*, *Asteraceae* (*Compositae*), *Fabaceae* (*Leguminosae*), *Oleaceae*, *Moraceae*, and *Thymelaeaceae*, respectively (Figure 3) [50]. The best known and researched coumarins in the field of phytochemistry, pharmacology, medicinal chemistry, and the food science can be found in these families. Therefore, these are the coumarins that are going to be further addressed in the next sections of this chapter.

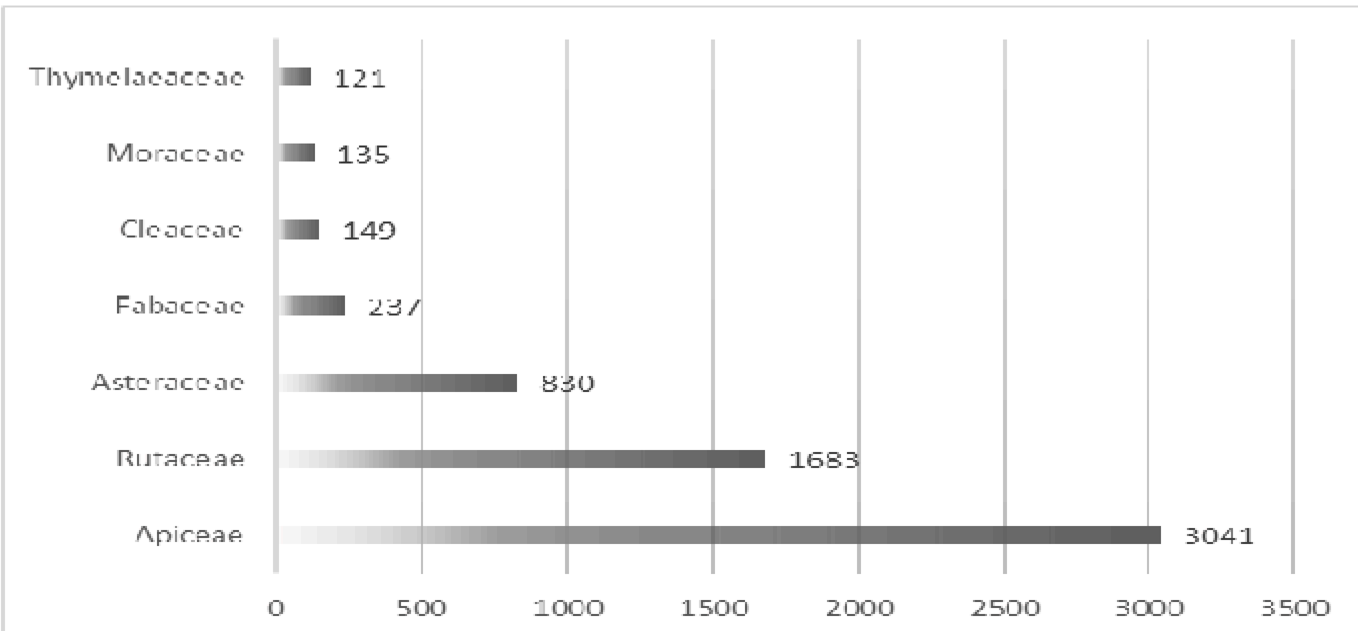

Figure 3.

Number of coumarins presented in seven different families of plants[50].

Coumarins usually are in the free state in plants as they are polar structures, and many of them can sublimate. They might also be found in the form of glycosides, including psoralen core-related structures [44]. They are characterized by UV light absorption, resulting in a very characteristic blue fluorescence; they are also very photosensitive as they can be altered by natural light [44]. These features are used in the isolation and analysis, as well as in unusual therapies such as photochemotherapy and the industry of chemical sensors [51, 52].

3. Biosynthesis of coumarins

Simple coumarins are biogenetically derived from shikimic acid, via cinnamic acid. The specificity of the process is the C-2 hydroxylation, producing a break (*β*-oxidation) of the side chain (i.e. *Salix* spp.), or chain isomerization and subsequent lactonization, generating the umbelliferone. Figure 4 explains the entire process [46, 53].

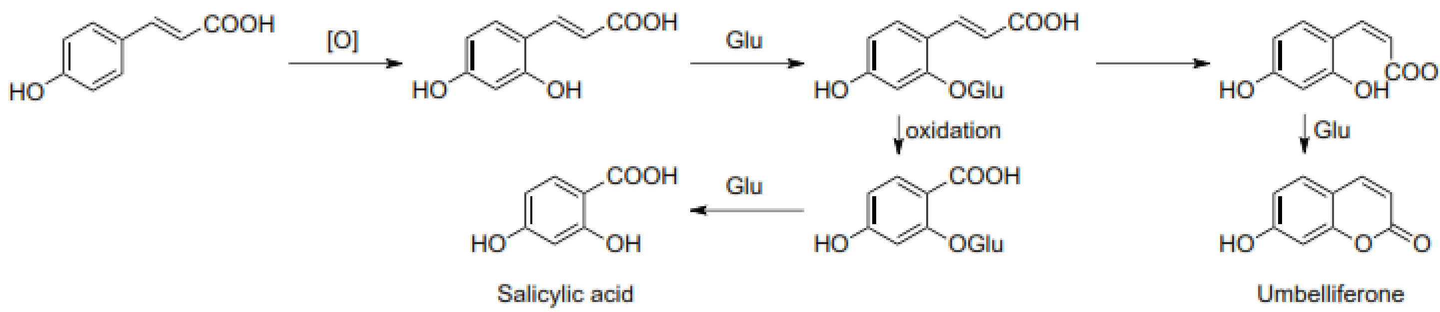

Figure 4.

Biosynthesis of simple coumarins.

Pyrano and furocoumarins (Figure 2) are also biogenetically derived from shikimic acid. These coumarins could be divided in two groups—lineal and angular—depending on the position where the isopentenyl pyrophosphate is condensed to further cyclize and form the heterocycle. The biosynthesis of these complex coumarins could also be the result of the cyclization of a simple coumarin previously prenylated [53].

Among the coumarins classified as "various" is the dicoumarol, which is formed by bacterial fermentation of Yellow Sweet Clover, and was isolated for the first time from decomposed leaves of *Melilotus albus* (*Fabaceae*/*Leguminosae*).

An approximation for the dicumarol biogenesis is the hydroxylation of the 4-position of the coumarin, that then captures a molecule of formaldehyde and is condensed with another molecule of 4-hydroxycoumarin, and finally enolize the keto group forming the dicumarol [46].

From a chemotaxonomic approach, Ribeiro & Kaplan (2002) evidenced that the diversity and structural complexity of the coumarins constitute an example of higher plant evolution. Simple coumarins are the most common in all angiosperms, especially in *Oleaceae* and *Asteraceae*, and their occurrence is of 100% and 98, 68%, respectively [50]. The second most prevalent coumarins are furocoumarins and pyranocoumarins. Coumarin in some families are high (*Thymelaeaceae*, *Rutaceae*, *Apiaceae*, *Fabaceae*, and *Moraceae*). In the case of well-diversified structural types in *Apiaceae* and *Rutaceae*, coumarins are considered as chemotaxonomic markers [50].

Apiaceae is the major source of coumarins (Figure 3) and one of the more diverse, containing five different types of coumarin derivatives (simple coumarins, lineal furocoumarins, angular furocoumarins, lineal pyranocoumarins, and angular pyranocoumarins) [50, 54]. *Rutaceae* is also highlighted in both occurrence and diversification. Generally, the division *Angiospermae* is preferably rich in simple coumarins, followed by the furo and pyranocoumarins [50].

4. Coumarins in medicinal plants

A large number of valuable species used commonly as medicinal plants, aromatic plants, and edible plants for human and animal feeding belongs to coumarin-rich plant families. Among them are species with well-documented biological activity, in which coumarins are part of the active principles. Table 1 shows a selection of plants of these families (first listed seven families with number of occurrence > 100) and some other families with species of particular pharmacological interest on chronic diseases. Coumarins presenting great pharmacological interest have been isolated in different geographical regions from other botanical families. Also shown are the coumarin compounds having species and their yield (if available).

Most of these plants are well known by people and scientists as part of herbal medicine repertories in Europe, Asia, or the Americas [55-58]. From the list, several coumarin-containing species or genera have also ethnomedical records in Cuba and the Caribbean Basin [59, 60]. Among of plant included are species with a great historical record of ethnomedicinal uses, and are present in *traditional medicine systems*: Ayurveda Medicine, Traditional Chinese Medicine and Unani Medicine, or in other recent cultures. Also, renowned species used on conventional therapeutics and modern herbal medicine are included, ie. *Aesculus hippocastanum* (Horse-chestnut), *Passiflora incarnata* (Passion Flower), *Lawsonia inermis* (Henna), *Hypericum perforatum* (Saint John Wort), *Tilia cordata* (Lime Tree) and *Uncaria tomentosa* (Cat's Claw).

Coumarins are also present in several species belonging to different botanical families, which are widespread in the northeastern region of Brazil [61]. Some of them are reported in folk medicine as traditional remedies drugs for the treatment of respiratory diseases [55]. Many pharmacological activities have been ascribed to coumarins such as anticlotting, hypotensive, antimicrobial, anti-inflammatory, and antitumor activities [61].

Recent studies and review manuscripts regarding the coumarin scaffold describe the huge variety of biological activities that may be present in the natural coumarins [8, 18, 62-64]. Venugopala et al. (2013) presented several coumarins displaying activities such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, anti-hypertensive, antitubercular, anticonvulsant, anti-adipogenic, Cytochrome P450 inhibiting, anti-hyperglycemic, antioxidant, and neuroprotective. Several recent reviews summarize and highlight advances in the application of coumarins, especially concerning their antioxidant and anticancer properties [62-70].From *Calophyllum* spp., it is remarkable the antiviral activity of calanolides and other related pyranocoumarins on Epstein-Barr virus and HIV [56]. As active compound of molluscicidal effects on *Biomphalaria glabrata* of *C. brasiliense* extracts were determined (-) mammea A/BB, also found in *C. Verticillatum* [71].

It is the great structural diversity of coumarinic compounds that allows for their several applications, and also allows for the high interest of these derivatives as phytochemicals. The pharmacological and biochemical properties and therapeutic applications of simple coumarins depend upon the pattern of substitution [68].

Family-species (vernacular name)	**Coumarin**	**Use***	**Reference**
Apiaceae/ Umbelliferae			

Family-species (vernacular name)	Coumarin	Use*	Referenc e
Ammi majus (*Bishop's flower*)	Imperatorin, bergapten, oxypeucedanin, pabulenol, marmesin, xanthotoxin, isopimpinellin and heraclenin.	M	[72]
A. visnaga (Pick-tooth, Toothpickweed)	PyranoCoumarins	M	[58, 73]
Anethum graveolens (Dill)	Aesculetin, bergapten, scopoletin	M, F	[60]
Angelica archangelica (Angelica)	Angelicin, osthol (major constituent in rhizome/root at 0.2%), bergapten, imperatorin, isoimperatorin (major constituent in fruit), oreoselone, oxypeucedanin, umbelliferone, xantonin, xanthotoxin, xanthotoxol	M, F	[57, 58]
Apium graveolens (Celery)	Apigravin, apiumetin, apiumoside, bergapten, celerin, celereoside, isoimperatorin, isopimpinellin, osthenol, rutaretin, seselin, umbelliferone, 8-hidroxy-5-methoxypsoralen.	M, F	[57, 58]
Coriandrum sativum (Coriander)	Umbelliferone,	M, F	[58]
Cuminum cyminum (Cumin)	Escopoloetina, bergapten	M, F	[58]
Daucus carota subsp. *carota* (Wild Carrot)	8-methoxypsoralen, 5-methoxypsoralen (0.01–0.02 ug/g) in fresh plant, concentration increased in the disease plant.	M	[57]
Foeniculum vulgare (Fennel)	Umbelliferone, esculetin, bergapten, seselin, psoralen	M, F	[58]
Ferula assafoetida (Asafoetida)	Umbelliferone, coumarin-sesquiterpene complexes e.g. asacoumarin A and asacoumarin B.	M	[57]
Petroselinum crispum (Parsley)	Bergapten and oxypeucedanin as major constituent (up to 0.02% and 0.01%, respectively); also 8-metoxypsoralen, imperatorin, isoimperatorin, isopimpinellin, psoralen, xanthotoxin (up to 0.003%).	M, F	[57, 58]
Pimpinella anisum (Aniseed)	Scopoletin, umbelliferone, umbelliprenine, bergapten	M, F	[57, 58]
Trachyspermum ammi /Carum copticum (Ajwain)	Coumarins	-	[74]
	Rutaceae		
Aegle marmelos (Bael fruit)	Sesquiterpenic coumarin ethers, diterpenic coumarin ethers, triterpenic coumarin ethers, sesterterpenic coumarin ethers, auraptene, epoxyauraptene, marmin.	M, F	[75-77]
Citrus aurantium (Bitter Orange tree)	Volatile Coumarins (0.09%): aurapteno, auraptenol, bergapteno, bergaptol, escoparona, citropteno.	M, F	[58]

Family-species (vernacular name)	Coumarin	Use*	Referenc e
C. limonum (Lemon tree)	Escopoletin, umbelliferone, bergamotin, bergapten, bergaptol, citropten	M, F	[58]
C. sinensis (Orange tree)	Herniarin, scopoletin	M, F	[60]
Melicope spp.	Coumarins, chromones, dichromones	M	[56]
Murraya paniculata (*M. exotica*) (Orange Jessamine, Chinese box)	Coumarins	M	[74]
Paramygnya monophylla	Poncitrin, nordentatin	M	[56]
Stauracanthus perforates	Coumarins	M	[78]
Tetradium daniellii (*Euodia daniellii*)	Coumarins	M	[79, 80]
Toddalia aculeata (*T. asiatica*) (Orange climber)	Ulopterol	M	[74, 81]
Zanthoxilum americanum (Northern Prickly Ash)	Xanthyletin, xanthoxyletin, alloxanthoxyletin, 8-(3,3-dimethylallyl)-alloxanthoxyletin.	M	[57]
Z. syncarpum	Coumarins	M	[82]
	Asteraceae/Compositae		
Achillea millefolium (Yarrow)	Coumarins (0.35%)	M	[58]
Ageratum conyzoides (Mexican ageratum)	1-2 benzopirone	M	[83]
Arnica montana (Arnica)	Scopoletin, umbelliferone	M, F	[57, 58]
Chamaemelum nobile (Roman Chamomile)	Scopoletin-7-glucoside	M, F	[57, 58]
Cichorium intybus (Chicory)	Coumarins	M, F	[73]
Conyza sumatrensis (Fleabane)	Osthol	M	[56]
Eupatorium triplinerve (White Snakeroot)	Coumarins	M	[84]
Hieracium pilosela (Mouse Ear)	Coumarins (0.2–0.6%): 7-glucosil-umbeliferone	M	[58]
Lactuca virosa (Wild Lactuce)	Aesculin, cichoriin	M	[58]
Matricaria recutita (Chamomille)	Umbelliferone and its methyl ether, heniarin.	M, F	[57, 58]
Mikania glomerata (Guaco)	Coumarins	M	[85]
Mikania hirsutissima	Coumarins	M	[86]
	Fabaceae/Leguminosae		
Dipteryx odorata (*Coumarouna odorata*) (Tonka Bean, Coumaru)	Coumarins (35,000 ppm)	M	[84]
Euchresta formosana	Coumarins	M	[87]
Medicago sativa (Lucerne)	Cumestrol, medicagol, sativole, trifoliole, lucernole, dafnoretin.	M, F	[57, 58]

Family-species (vernacular name)	**Coumarin**	**Use***	**Referenc e**
Melilotus officinalis (Yellow Sweet Clover)	Coumarins (0.4–1%)	M	[84] [58]
Glycyrriza glabra (Liquorice)	Glycyrin, heniarin, liqcoumarin, umbelliferone, GU-7 (3-arylcoumarin derivative)	M, F	[57, 58]
Myroxylon balsamum (Balsam Tolu)	Coumarins	M	[58]
Trigonella foenum-graecum (Fenugreek)	Coumarins	M, F	[57, 58]
Moraceae			
Dorstenia brasiliensis	Coumarins	M	[88]
Morus alba (White Mullberry)	Coumarins	M	[89]
Oleaceae			
Fraxinus excelsior (Common ash)	Fraxoside, esculoside, fraxinol, escopoletoside	M	[57]
Oleae europaea (Olive)	Coumarins	M, F	[90]
Thymelaeaceae			
Daphne feddei	feddeiticin (dicoumarinolignoid), dicoumarin glucosides	-	[91]
D. gnidium (Flax-leaved daphne)	daphnetin, daphnin, acetylumbelliferone, daphnoretin	-	[92]
D. odora (Winter daphne)	daphnetin	-	[93]
D. oleoides	dimeric coumarin glycoside, trimeric coumarin fucosides, daphnetin,	-	[94, 95]
D. pedunculata	3-[(3-hydroxy-4-ethylpropanpicatephenyl)oxy] -6-methoxy-7-hydroxycoumarin	-	[96]
Achanthaceae			
Justicia pectoralis (Tilo)	Coumarin, umbelliferone	M	[97]
Araliaceae			
Eleutherococcus senticosus (Eleutherococcus)	Coumarins	M	[58]
Brassicacae/Cruciferae			
Radicula armoracia (Horseradish root)	Aesculetin	M, F	[57, 58]
Caryophylacae			
Herniaria glabra (Rupture wort)	(0.1-0.4%) umbelliferone, herniarin	M	[58]
Caprifoliaceae			
Vivurnum prunifolium (American black haw)	Scopoletin (7-hidroxy-6-methoxicoumarin), scopolin, sculetin	M	[58]
Clusiaceae/Guttiferae			
Calophyllum brasiliense (Guanandi, Ocuje)	volatile Coumarins, (-) mammea A/BB, brasimarins A, B, and C		[71, 98]

Family-species (vernacular name)	**Coumarin**	**Use***	**Reference**
C. cerasiferum	(-) calanolide B	M	[56]
C. cordato-oblongum	Coumarins	M	[56]
Calophyllum inophyllum (Borneo mahogany)	Coumarins		[56]
Calophyllum lanigerum var *austrocoriaceum*	(+)- calanolide A	M	[56]
C. teysmannii var *inophylloide*	(-) calanolide B, sonlattrolide	M	[56]
C. verticillatum	mammea A/BB		[71]
Connaraceae			
Connarus monocarpus	Bergenin1.5%	M	[56]
Cupresaceae			
Juniperus communis (Common Juniper)	Umbeliferone	M	[58]
Hippocastanacae			
Aesculus hippocastanum (Horse-chestnut)	Aesculetin, fraxin, scopolin, aesculetosides (glucosides)	M	[57, 58]
Hypericaceae			
Hypericum perforatum (Saint John Wort)	Umbelliferone, escopoletin,	M	[58]
Lamiaceae/Labiadae			
Lavandula angustifolia (Lavender)	Coumarins: 1,500 ppm, 0.25%: hernairin, santonin	M	[58, 84]
L. latifolia (Aspic)	Coumarins: 22 ppm	M	[84]
Lycopus europeus (European Bugle)	Coumarins: 1,200 ppm	M	[84]
Ocimum basilicum (Basil)	Aesculetin, aesculin	M, F	[60]
Salvia officinalis (Garden Sage)	Esculetin	M	[58]
Lauraceae			
Cinnamomum cassia (C. aromaticum) (Chinese cinnamon)	Coumarins	M, F	[57]
C. verum (*C. zeylanicum*) (Cinnamon)	Coumarins (0.65%)	M, F	[57, 58]
Laurus nobilis (laurel, sweet bay)	Coumarins	M, F	[57]
Persea americana (Avocado)	Scopoletin	M	[60]
Lytraceae			
Lawsonia inermis (Henna)	Coumarins	M	[58]
Meliaceae			
Trichilia hirta (Guabán)	Coumarins	M	[99]
Menianthaceae			
Menyanthes trifoliata (Buckbean)	Scoparone, brailin, scopoletin	M	[58]
Monimiaceae			

Family-species (vernacular name)	Coumarin	Use*	Reference
Peumus boldus (Boldus)	Coumarins 125 ppm	M	[84]
	Passifloraceae		
Passiflora incarnate (Passion Flower)	Scopoletin, umbelliferone	M, F	[57, 58]
	Plantaginaceae		
Plantago major (Large Plantain)	Esculetin	M	[58]
	Poaceae (Graminae)		
Zea mays (Corn)	Coumarins: 2,000 ppm	F	[84]
	Rhamnaceae		
Zizyphus jujube (Jujube)	Coumarins: 3,000 ppm	M, F	[84]
	Rubiaceae		
Galium odoratum (*Asperula odorata*) (Woodruff)	Coumarins: 13,000 ppm	M	[84]
Uncaria tomentosa (Cat's Claw)	Coumarins	M	[100]
	Tiliaceae		
Tilia cordata (Lime tree)	Fraxosides, sculosides	M	[58]
	Urticaceae		
Urtica dioica (Nettle)	Scopoletin	M, F	[57, 58]

Table 1.
Medicinal and food plant uses of some species from major coumarin-containing families.
* M: Medicine, F: Food.

6. Extraction techniques and identification of coumarins

There have been a variety of methods described for extraction of coumarins. Generally, coumarins extraction can be performed either on dry or fresh material, with solvents of different polarities, depending on the type of structure. Some coumarins are sparingly soluble in apolar solvents and often they can be crystallized directly by cooling or concentrating the solvent.

Miranda and Cuéllar (2001), in their book entitled "Farmacognosia y Productos Naturales ", raised the possibility of following four variants using the Soxhlet method:

- Soxhlet extracts the dry powdered material with petroleum ether continuously for 3 days. The ether extract is concentrated until 1/5 of the original volume and it is cooled to obtain the crystallization of the extract. Coumarins are presented in the obtained solid.
- The dry material is removed and sprayed with ethanol, continuously using the Soxhlet for 2–3 days, at least. The extract is concentrated under vacuum to an oily residue. This residue is repeatedly washed with portions of hot water. The aqueous washings are combined and concentrated to the minimum volume, acidifying with hydrochloric acid solution 10%. The mixture is refluxed for 30 minutes. If some precipitate appears, it is

filtered (hot filtration) and the solution is allowed to cool. The crystals are collected by filtration and found therein the coumarins.
- Available materials are extracted with ethyl ether or by successive macerations with the Soxhlet apparatus. The extract is concentrated to dryness and the residue contains the coumarins.
- The dried and ground plant material is extracted with acetone continuously in a Soxhlet apparatus, for at least 3 days, and the extract is concentrated to dryness giving a residue that contains the coumarins.

Purification and separation of coumarins contained in various extracts could be performed by using chromatographic columns, using as a carrier aluminum oxide and as solvent the eluotropic series: benzene-hexane (1:2.5); benzene; chloroform; chloroform-acetone, in proportions of a linear gradient to pure acetone [44]. For recognition of the described structures some trials were described, within which there are:

- Those that recognize coumarin’s phenolic substitutions where Emerson's Reagent is used, developing color.
- The presence of lactone groups can be observed leading to changes of pH in the medium. When coumarins are dissolved in ethanol, solutions change the color when acidified (yellow color disappears).
- The furan ring can be recognized by using the Erlich test. The extract is treated with a solution of dimethylamino-benzaldehyde (5% ethanol), and then acidified by bubbling gaseous hydrochloric acid. The orange color indicates a positive test.

The last test is commonly used for phytochemical screening that is initially performed in plant research. Carrying on this approach, Payo et al., (1996), from 39 Cuban species screened, detected that 51.2% were positive for coumarin test.

Other extraction methods used in coumarins are: microwave, sonication, and supercritical fluid extraction (SFE) [136], these tests also propose capillary electrophoresis for natural products isolation.

Therefore, on the isolation and analysis of coumarins diverse methods have been used: chromatography (paper chromatography, thin layer chromatography, gas chromatography, and high-performance liquid chromatography), titrimetric, and spectrophotometric (colorimetric and polarographic) methods [1].

Due to coumarin-characteristic chromophore groups and its strong UV absorption at around 300 nm, it is routinely possible to be detected by feasible methods such as ultraviolet–visible spectroscopy (UV–vis) [137]. UV–vis detector is used in high performance liquid chromatography (HPLC) and also other hyphenated techniques are employed to characterize and quantify natural products such as Liquid Chromatography (LC)–Photo Diode Array Detector (PDA), coupling of Mass Spectrometry to LC–[137], or Ultra Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS) [138].

A simple spectroscopic technique [35] and HPLC [85] were employed to determine coumarins in the Brazilian medicinal plant "guaco", *Mikania glomerata* (*Asteraceae*). Coumarin HPLC detection is also used in Cuba to standardize a sedative herbal medicine based on *Justicia pectoralis* (*Acathanceae*) [97, 139].

7. Conclusion

Coumarins have been increasingly attracting special interest as phytochemicals due to their underlying outstanding contributions in the prevention and treatment of diseases. Coumarins represent a diverse class of phytochemicals that are ubiquitous in the human diet. Some of the medicinal usages of extracts of plants containing coumarins have been proven in experimental models, which suggested that the extracts possess various pharmacological actions. Several related researches and developments make coumarins an extremely attractive scaffold. The role of coumarins as important phytochemicals and their interesting applications were presented and discussed in this book chapter. The origin, natural sources, biosynthesis, and applications were also described