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Mini-Review on Coumarins: Sources, Biosynthesis, Bioactivity, Extraction and Toxicology

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Abstract: Coumarins are a class of naturally occurring compounds found in various plants, fungi, and microorganisms, each with a unique chemical profile. These compounds exhibit a broad range of bioactivities, including antithrombotic, anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer, and neuroprotective properties. The effective extraction of coumarins, facilitated by methods such as maceration and microwave-assisted extraction, is integral to unlocking their potential across various applications. Nevertheless, safety and toxicology considerations assume paramount importance, particularly in pharmaceuticals, cosmetics, and food additives. While moderate dietary consumption of coumarin-rich foods is generally safe, excessive intake, whether through foods or supplements, raises concerns linked to hepatotoxicity and photosensitivity. Notably, specific coumarin derivatives, including the widely used anticoagulant warfarin, necessitate precise dosing and vigilant monitoring to mitigate the risk of bleeding complications. In conclusion, the versatile biological activities of coumarins underscore their significance; yet, their safety and toxicity profiles are contingent on multiple factors, encompassing compound type, dosage, and individual susceptibility. This review provides a holistic understanding of coumarins, encompassing their natural origins, biosynthesis, bioactivity spectrum, extraction techniques, and insights into safety, and toxicology.

Keywords: Coumarins, Natural Sources, Biosynthetic Pathway, Extraction, Toxicology.

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1. INTRODUCTION

Coumarins, a class of natural compounds characterized by their benzopyrone structure, have been the subject of significant scientific interest and research due to their widespread occurrence in nature and diverse biological activities (1). These compounds, originally identified in tonka beans (*Dipteryx odorata*) and later found in various plants, fungi, and microorganisms, have demonstrated a wide range of pharmacological properties, making them intriguing candidates for applications in pharmaceuticals, food additives, cosmetics, and beyond (2).

The intriguing chemical structure of coumarins consists of a benzene ring fused to an α-pyrone (benzopyrone) ring, creating a scaffold with distinct electronic and steric properties. This structural arrangement confers upon coumarins a remarkable ability to interact with a myriad of biological targets, resulting in their diverse bioactivity spectrum (3). The presence of coumarins in edible plants and their established usage in traditional medicine practices have long hinted at their potential health benefits (4).

Natural sources of coumarins are abundant and encompass a wide array of botanical species. Plants such as cinnamon, sweet woodruff, and sweet clover are renowned for their coumarin content. Coumarins are distributed across various plant parts, including flowers, seeds, and leaves, with distinct classes such as pyranocoumarins and furanocoumarins contributing to their molecular diversity (5). Fruit and flowers often exhibit higher coumarin concentrations compared to other plant parts, rendering them noteworthy sources for isolation and analysis (6).

Beyond plants, coumarins have also been identified in microorganisms, further expanding their biogeographical distribution. Fungi, in particular, have yielded coumarin derivatives from species isolated in diverse ecological niches (7). These coumarins, typically encountered as secondary metabolites in plants and microorganisms, serve essential functions in protecting against various environmental threats, including herbivores and pathogens (8).

The pharmacological importance of coumarins is undeniable. These compounds have demonstrated a myriad of bioactivities, including antithrombotic, anti-inflammatory, antioxidant, antimicrobial, anti-viral, anticancer, and neuroprotective effects (1). Such a versatile pharmacological profile has spurred extensive research into their potential therapeutic applications. Coumarins have found utility in the treatment of various medical conditions, with some derivatives serving as anticoagulants to prevent blood clot formation (9). Additionally, their antioxidant properties make them attractive candidates for the development of novel therapies aimed at mitigating oxidative stress-related diseases (10).

In the context of food and cosmetics, coumarins have been explored as natural additives and functional ingredients. Their fragrance and flavor-enhancing properties have led to their incorporation into perfumes, and food products (11). Furthermore, coumarins have shown potential in the cosmetic industry due to their skin-lightening and antioxidant effects (12).

While coumarins offer remarkable promise, they are not without their challenges and risks. Excessive consumption of coumarin-rich foods or dietary supplements has raised concerns regarding potential adverse effects, including hepatotoxicity and photosensitivity (13). The delicate balance between their beneficial bioactivities and potential health risks underscores the need for a comprehensive understanding of coumarins' safety and toxicology profiles.

This mini-review endeavors to provide an overview of coumarins, encompassing their natural sources, biosynthesis, bioactivities, extraction methods, and safety considerations. We aim to shed light on the multifaceted world of coumarins, offering insights into their potential applications, while emphasizing the importance of responsible use and vigilant monitoring to harness their benefits effectively.

2. NATURAL SOURCES OF COUMARINS

Coumarin is present in numerous vegetables, fruits, spices, and herbs, as illustrated in Figure 1. Within plants, coumarins are distributed across various plant parts, including flowers, seeds, and leaves (5). They exhibit varying concentrations and molecular diversities, with distinct classes such as pyranocoumarins (linear and angular) and furanocoumarins (linear and angular) (5). Generally, fruit and flowers contain higher coumarin concentrations compared to roots, stem bark, leaves, and seeds (6). Table 1 offers an overview of coumarin and its derivative classes, their isolation from various plant sources, as well as pertinent details such as the source parts and isolation methods, along with the number and types of coumarins identified.

In addition to plants, coumarin and its derivatives have been discovered in microorganisms. In a 2015 study, J. Wang et al. reported the isolation of a novel coumarin derivative from two fungal species found along the shores of the South China Sea, which they subsequently co-fermented (14). Similarly, Uma-shankar et al. published a study in the same year where they extracted three coumarin compounds from Alternaria mushrooms isolated from the leaves of Crotalaria pallida (15). In a 2020 investigation, a new coumarin derivative was identified, along with pyron-derived compounds, in the Aspergillus versicolor fungal strain isolated from Coridius chinensis (16). These coumarins, typically encountered as secondary metabolites in plants and microorganisms, serve a critical function in defending against various threats (6).

3. BIOSYNTHESIS OF COUMARINS

Plants synthesize many phenolic compounds (37). These synthesized compounds are classified into four distinct groups according to their carbon scaffolds: 1) phenolic acids (C6-C1), 2) hydroxycinnamic acids (HCs, C6-C3), 3) stilbenes (C6-C2-C6), and 4) flavonoids (C6-C3-C6). The hydroxycinnamic acids class includes p-coumaric acid, caffeic acid, and ferulic acid (38). Phenolic compounds, including p-coumaric acid, caffeic acid, and ferulic acid, are synthesized in plants from phenylalanine or tyrosine through the activity of phenylalanine ammonia lyase (PAL) or tyrosine ammonia lyase (TAL) enzymes. The biosynthesis of coumarins, as depicted in Figure 2, involves the conversion of phenylalanine and tyrosine using PAL or TAL, followed by enzymatic reactions catalysed by 4-cinnamic acid: coenzyme A ligase (4CL) and feruloyl CoA 6’-hydroxylase (F6’H) (also known as 2-oxoglutarate-dependent dioxygenase, 2OGD, or p-coumaryol CoA 2’-hydroxylase, C2’H) enzymes (37,38). Following deamination by PAL, the initial enzyme in the phenylpropanoid pathway, cinnamic acid is produced and subsequently hydroxylated by cinnamate 4-hydroxylase (C4H) to yield p-coumaric (4-coumaric) acid (39,40). p-Coumaric acid serves as a precursor for coumarin derivatives, including umbelliferone, catalysed by 4CL and F6’H enzymes, and esculetin, which is produced with the participation of coumarate-3-hydroxylase (C3H), 4CL, and F6’H enzymes (38,39). p-Coumaroyl-CoA can be converted to esculetin through a series of enzymatic reactions, starting with hydroxycinnamoyl transferase (HCT) and followed by C3H and HCT enzymes, respectively (41–44). Caffeic acid, derived from the hydroxylation of p-coumaric acid by the C3H enzyme (45), can further undergo

O-methylation via caffeic acid O-methyl transferase (COMT) to produce ferulic acid (38). Ferulic acid is subsequently transformed into scopoletin, one of the coumarin derivatives, through the actions of 4CL and F6'H enzymes (46). The formation of feruloyl-CoA, an intermediate in the conversion of ferulic acid to scopoletin, can also occur from caffeoyl-CoA via caffeoyl-CoA O-methyltransferase (CCoAOMT) (47).

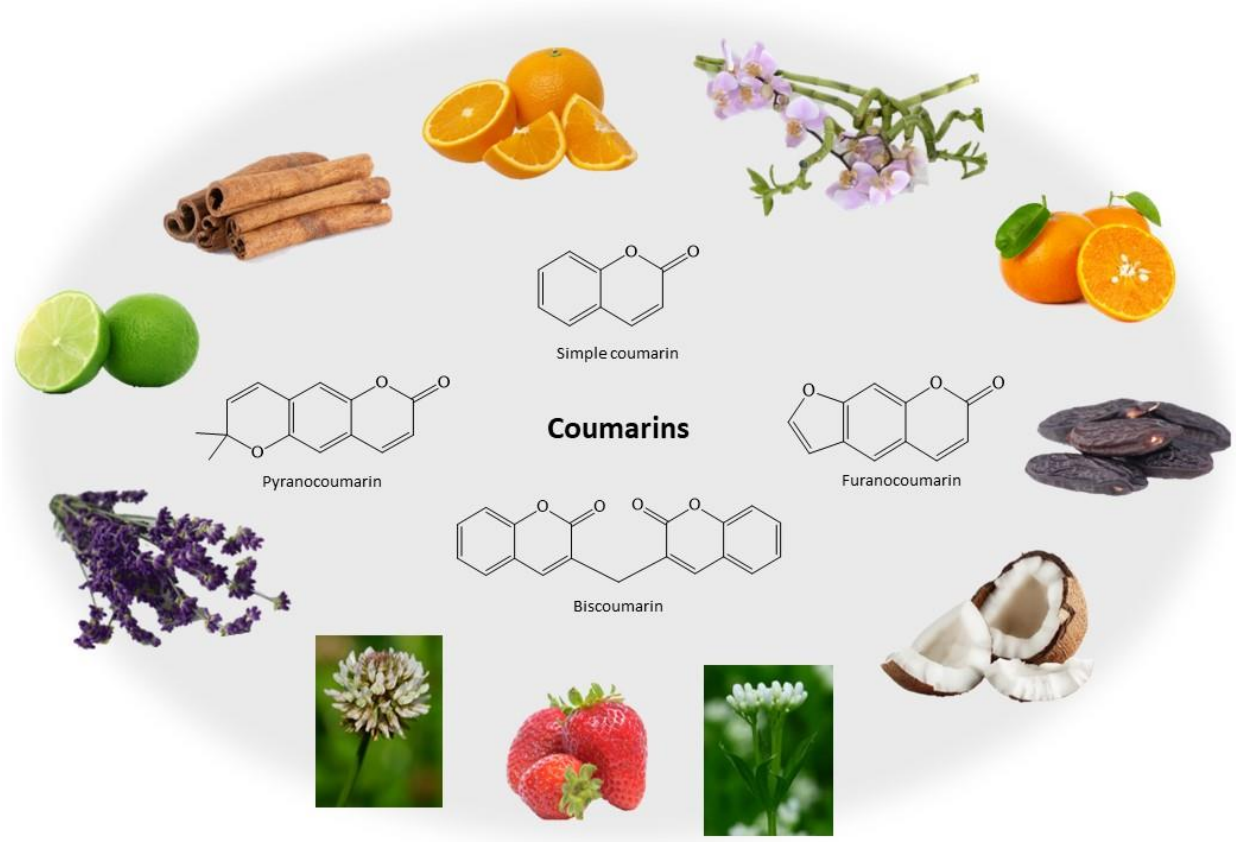

Figure 1: The structure and natural sources of various coumarin classes.

While the conversion of phenylalanine-derived coumaric acid to coumarin has been reported to occur through a single-step ortho-hydroxylation process, except for the para- hydroxylation, this biosynthetic step remains relatively underexplored (48). An alternative pathway for coumarin biosynthesis begins with the conversion of phenylalanine-derived coumaric acid into trans-2-cinnamate by the enzyme cinnamate 2-hydroxylase (C2H) and proceeds through subsequent transformations catalyzed by 2-coumarate O-β-glucosyltransferase (2GT) and β-glucosidase (GBA) (49).

Despite plants' capacity to synthesize various flavonoid compounds, including coumarins, their natural extraction is often insufficient for applications such as drug development. Traditional tissue culture and chemical synthesis methods prove impractical for large-scale coumarin production (37). Consequently, researchers have turned to microorganisms as an alternative for biosynthesizing phenolic compounds, including coumarins (40,50). These studies involve the transfer of structural genes isolated from plants, fungi, and bacteria into microorganisms like *Escherichia coli* and Saccharomyces cerevisiae, resulting in recombinant microbial strains capable of producing phenolic compounds (37). In 2015, Yang et al. achieved the synthesis of coumarin derivatives using *E. coli*. They cloned and transferred Os4CL and IbF6'H2 genes from *Oryza sativa* and *Ipomoea batatas* into *E. coli*. This genetic modification enabled the *E. coli* to produce three coumarin derivatives—umbelliferone, esculetin, and scopoletin—when cultured in media supplemented with hydroxycinnamic acids such as p-coumaric acid, caffeic acid, and ferulic acid (38).

Table 1: Classes and Isolation of Plant-Derived Coumarins.

Sources	Part Of Source	Isolation Method	Solvent	Temperature	Time	Coumarins Number and Type	Ref.
***Ailanthus altissima* (Mill.) Swingle**	Air-dried root barks	-	70% EtOH-H_2O	70 °C	3 h	14 compounds (Simple coumarins)	(17)
Angelica dahurica	Air-dried roots	Reflux	95% EtOH-H_2O	-	3 h	23 compounds (10 furanocoumarins + 3 furanocoumarin dimers)	(18)
11 species of Bamboo	Leaves	Ultrasound-Assisted Extraction + Centrifugation	70% EtOH-H_2O and MeOH	RT and 4°C	30 min	12 compounds (8 simple coumarins + 4 furanocoumarins)	(19)
Bombax ceiba	Dried flowers	-	MeOH and 70% MeOH	55 °C and RT (20 °C)	5 + 20 h	24 compounds (4 simple coumarins + 13 flavones + 3 glycosides + 4 phenolic acids)	(20)
Calophyllum brasiliense	Air-dried leaves	-	Hexane	RT	-	3 compounds (Simple coumarins)	(21)
Calophyllum inophyllum	Air-dried leaves	-	95% EtOH-H_2O	-	-	6 compounds (Pyranocoumarins)	(22)
5 species of Citrus	Peels	Percolation	MeOH	RT	5 days	6 compounds (2 simple coumarins + 4 furanocoumarins)	(23)
6 species of Citrus	Dried peels	Centrifugation	80% MeOH-H_2O	RT	~1.5 h	27 compounds (6 simple coumarins + 21 furanocoumarins)	(24)
***Citrus grandis* (L.)**	Dried fruits	Reflux	70% EtOH-H_2O	-	1.5 h	17 compounds (10 simple coumarins + 7 furanocoumarins)	(25)
Clausena excavate	Air-dried roots	Reflux	95% EtOH-H_2O	-	-	9 compounds (pyranocoumarins)	(26)
***Cuphea ignea* (Lythraceae)**	Fresh whole plant	Reflux	Hot EtOH/H2O (3:1)	-	8 h	1 compound (Simple coumarins)	(27)
Ferula sinkiangensis	Seeds	Reflux	95% EtOH-H_2O	-	2 h	11 compounds (Simple coumarins)	(28)
Ferulago subvelutina	Milled roots	Percolation	Ethyl acetate	RT	48 h	6 compounds (2 simple coumarins + 4 furanocoumarins)	(29)

***Matricaria chamomilla* (L.)**	Leaves	-	EtOH	-	-	7 compounds (5 simple coumarin + 2 cinnamic acid derivatives)	(30)
***Melilotus officinalis* (L.)**	Air-dried flowering tops	Soxhlet, Ultrasound-Assisted Extraction, Microwave-Assisted Extraction	95% and 50% EtOH-H_2O	-	8 h	3 compounds (1 Simple coumarin + 2 phenolic acid)	(31)
***Melittis melissophyllum* L.**	Air-dried and fresh leaves	-	80% MeOH	RT	30 min	1 compound (o-coumaric acid glucoside)	(32)
***Murraya paniculate* (L.) Jack**	Air-dried leaves and stems	-	95% and 50% EtOH-H_2O	-	-	19 compounds (Simple coumarins)	(33)
Paxillus involutus	Dried fruiting bodies	-	Ethyl acetate	4°C	~ 4 days	4 compounds (1 furanocoumarins)	(34)
Trifolium repens	Flowers	Soxhlet	Petrol and $CHCl_3$ + MeOH and 70% MeOH	RT	8 + 8 h	5 compounds (2 simple coumarins + 3 biscoumarins)	(35)
Zanthoxylum schinifolium	Freeze dried leaves	-	80% MeOH	-	-	9 compounds (Simple coumarins)	(36)

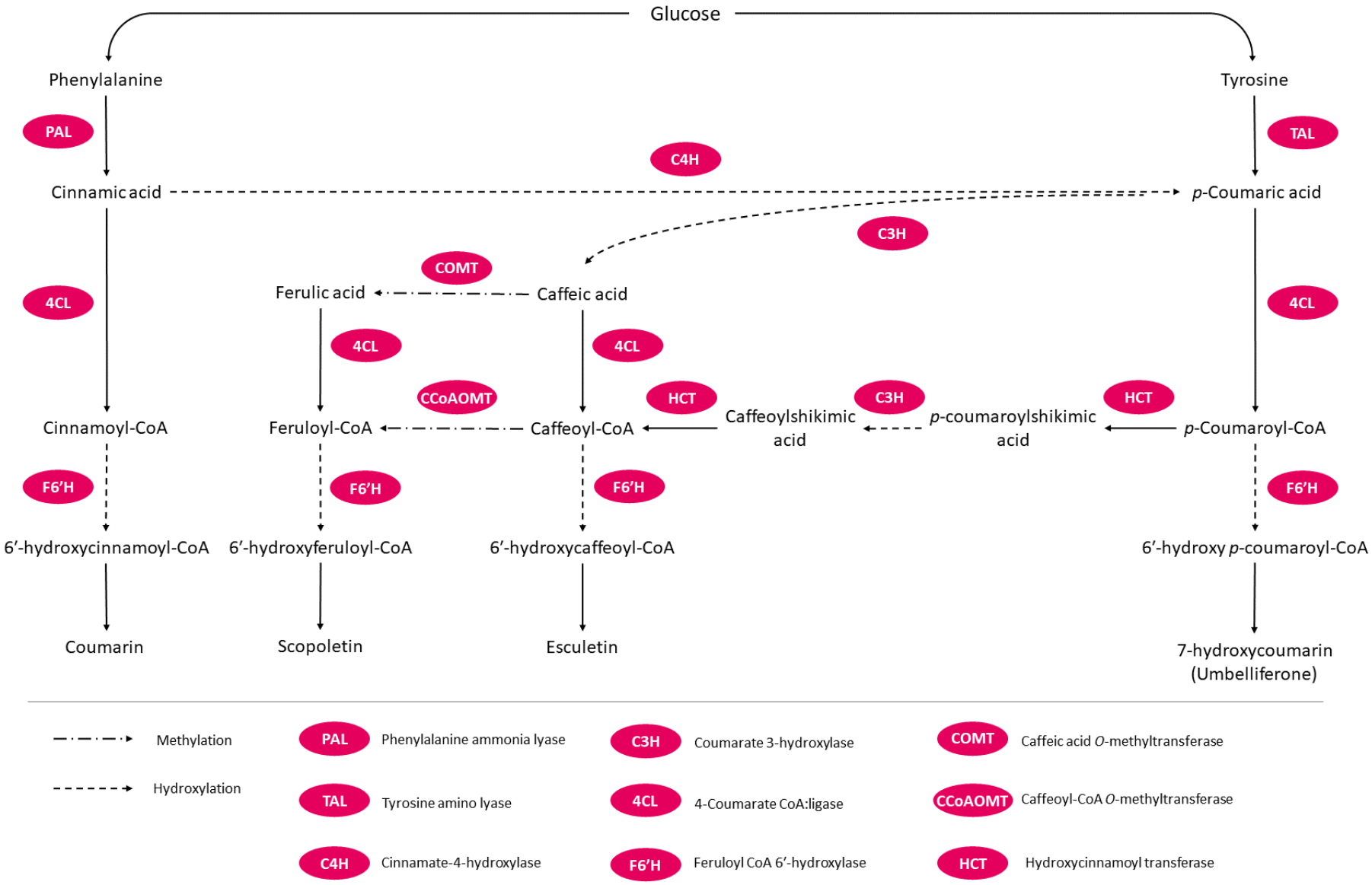

Figure 2: Biosynthesis Pathway of Coumarins.

4. BIOACTIVITY OF COUMARINS

Coumarins, a class of naturally occurring compounds found in various plants, fungi, and microorganisms, have garnered significant attention due to their diverse and promising biological activities (51). These compounds exhibit a wide range of pharmacological properties, making them subjects of extensive research for potential therapeutic applications (2). Below, we delve into some of the notable bioactivities associated with coumarins:

Antithrombotic Activity: One of the well-documented bioactivities of coumarins is their antithrombotic (anticoagulant and antiplatelet) activity (52). The prototypical coumarin derivative, warfarin, has long been used as an oral anticoagulant to prevent blood clot formation. It functions by inhibiting vitamin K epoxide reductase, a key enzyme in the coagulation cascade (53). This property has led to the development of various coumarin-based anticoagulant drugs.

Anti-Inflammatory Properties: Several coumarin compounds have demonstrated anti-inflammatory activity, which can be attributed to their ability to inhibit pro-inflammatory enzymes and cytokines (54). Coumarin derivatives, such as scopoletin and herniarin, have shown potential in attenuating inflammatory responses, making them of interest in conditions characterized by excessive inflammation (10,55).

Antioxidant Effects: Many coumarins exhibit potent antioxidant properties, which can help protect cells and tissues from oxidative damage caused by reactive oxygen species (ROS) (56). These antioxidants scavenge free radicals and reduce oxidative stress, potentially contributing to the prevention of various chronic diseases (57).

Antimicrobial Activity: Coumarins have displayed antimicrobial activity against a wide range of microorganisms, including bacteria (58), fungi (59), and parasites (60). Some coumarin derivatives have been investigated for their antibacterial and antifungal properties, suggesting their potential as natural antimicrobial agents.

Antiviral Activity: Several studies have investigated the effects of coumarin derivatives on various viruses, highlighting their promising role in combating viral infections. Some coumarin derivatives have exhibited the ability to inhibit the replication of certain viruses. This inhibition can occur through different mechanisms, including interference with viral RNA or DNA synthesis, disruption of viral protein function, or inhibition of viral entry into host cells. Coumarins have shown efficacy against a range of viruses, including but not limited to hepatitis virus, Influenza Virus, and HIV (61).

Anticancer Potential: Emerging research has highlighted the anticancer potential of certain coumarins. These compounds have shown cytotoxic effects on cancer cells and may inhibit tumor growth by various mechanisms, including apoptosis induction and cell cycle arrest (62).

Neuroprotective Effects: Coumarins, such as esculetin and osthole, have exhibited neuroprotective properties by modulating neuroinflammatory responses and protecting neurons from oxidative stress (63). These findings raise the possibility of coumarin-based treatments for neurodegenerative disorders (64).

In addition to the mentioned bioactivities, coumarins have also been investigated for their potential in managing various conditions such as diabetes and cardiovascular diseases (65,66). Their versatile pharmacological profile continues to drive research into their therapeutic applications (2). It is important to note that the bioactivity of coumarins can vary widely depending on their chemical structure, concentration, and the biological context. Further research is necessary to elucidate the specific mechanisms of action and clinical potential of different coumarin derivatives.

5. EXTRACTION METHODS

The efficient extraction of coumarins from natural sources is a critical step in harnessing their potential for various applications, including pharmaceuticals, food additives, and cosmetics (5). The choice of extraction method plays a pivotal role in determining the yield and purity of the extracted coumarins. Below, we explore several extraction techniques commonly employed to isolate coumarins from their natural matrices:

Reflux Method: The reflux method involves heating a mixture of the coumarin-rich source material and solvent in a round-bottomed flask fitted with a condenser. The solvent vaporizes and condenses, creating a continuous cycle. This refluxing process facilitates the extraction of coumarins as the solvent repeatedly comes into contact with the source material. After refluxing for a specified time, the extract is collected (67).

Soxhlet Extraction: Soxhlet extraction is a continuous extraction technique that involves solvent reflux. The raw material is placed in a porous thimble, which is inserted into a Soxhlet extractor. Solvent is continuously boiled, evaporating and condensing in a reflux system. The condensed solvent drips back onto the material, ensuring prolonged contact and efficient extraction. Soxhlet extraction is suitable for coumarins in samples with relatively low coumarin content (68).

Hydrodistillation Method: Hydrodistillation is a method used primarily for extracting essential oils, including those containing coumarins. In this method, steam is passed through the source material, vaporizing compounds. The steam and extracted compounds are condensed and collected

separately, with the essential oil enriched in coumarins obtained as the distillate (69).

Maceration: Maceration is a simple and widely used extraction technique, particularly suitable for extracting coumarins from dried plant material. In this method, the coumarin-rich source is soaked or immersed in a solvent at room temperature for an extended period (at least 3 days). During maceration, the solvent gradually absorbs the coumarins from the plant material. The resulting extract is then filtered to remove solid particles, and the solvent is evaporated to obtain the coumarin-rich extract. Maceration is a relatively gentle method and is especially useful for heat-sensitive compounds (68).

Infusion Method: The infusion method involves soaking the coumarin-rich source material in a suitable solvent, typically at room temperature or slightly elevated temperatures. The solvent gradually absorbs the coumarins over time. After the desired extraction period, the mixture is filtered, and the solvent is evaporated to obtain the coumarin-rich infusion (68). This method is similar to the maceration method but differs in the duration and temperature of the solvent.

Percolation Method: Percolation represents a continuous extraction technique that involves the passage of a solvent through a densely packed bed of coumarin-rich material. This method employs specialized equipment known as a percolator, typically a narrow, cone-shaped glass vessel open at both ends. To initiate the process, finely powdered plant material is moistened with the chosen extraction solvent. Subsequently, an additional quantity of solvent is introduced, and the mixture is allowed to rest. Following this, the content is transferred into the percolator and allowed to stand for 24 hours. This process results in the gradual dissolution of coumarins. Finally, the outlet of the percolator is opened, and the liquid contained therein is allowed to drip slowly. The resulting extract is then separated through filtration (68,70). This method is often used for both small and large-scale extraction processes and allows for precise control of solvent flow rates and extraction times (71).

Supercritical Fluid Extraction (SFE): Supercritical fluid extraction is an advanced method that uses supercritical fluids, such as carbon dioxide (CO_2), to extract coumarins. Under specific temperature and pressure conditions, CO_2 becomes a supercritical fluid with unique solvation properties (72). It can selectively dissolve coumarins from the raw material, and upon depressurization, the coumarins precipitate, leaving behind a concentrated extract. SFE is considered environmentally friendly and offers precise control over extraction parameters (73).

Microwave-Assisted Extraction (MAE): Microwave-assisted extraction is a rapid and efficient method for coumarin extraction. In MAE, the sample is exposed to microwave radiation in the presence of a suitable solvent. The microwave energy accelerates the extraction process by promoting the release of coumarins from the plant matrix. This method is known for its shorter extraction times and improved efficiency (68).

Ultrasonic-Assisted Extraction (UAE): Ultrasonic-assisted extraction employs high-frequency ultrasound waves to enhance the extraction process. The cavitation generated by ultrasound disrupts cell walls and facilitates the release of coumarins into the solvent (70). UAE is known for its shorter extraction times, reduced solvent consumption, and improved extraction efficiency (74).

In a comparative study conducted by Molnar et al., it was found that the maceration method was at least five times more efficient than the Soxhlet, hydrodistillation, and supercritical CO2 extraction methods and provided the highest umbelliferone and herniarin extraction (75). In a study by Chanfrau et al., reflux and Ultrasonic-Assisted Extraction methods were compared. As a result of the study, it was found that reflux provides a higher coumarin yield, and the use of low-frequency ultrasound in the extraction method provides a higher coumarin yield than high-frequency ultrasound (74).

As a result, the choice of extraction method should consider factors such as the nature of the source material, the desired coumarin compounds, and the specific requirements for purity and yield. Researchers often optimize extraction conditions, including solvent type, temperature, and extraction time, to maximize the recovery of coumarins while minimizing undesirable co-extracts. Ultimately, the selected method should align with the intended application of the extracted coumarins.

6. SAFETY AND TOXICOLOGY

The safety profile of coumarins has garnered significant interest, given their widespread presence in nature and their versatile applications in pharmaceuticals, food additives, and cosmetics (2). While coumarins offer diverse bioactivities, their consumption and exposure demand careful consideration to mitigate potential health risks.

Coumarins are naturally occurring compounds found in various edible plants like cinnamon, tonka beans, and certain fruits. In these natural forms, coumarins are generally deemed safe when consumed in typical dietary quantities (76). However, excessive consumption of coumarin-rich foods or dietary supplements can raise concerns regarding cumulative exposure (5). While natural coumarins may offer potential health benefits, excessive intake can lead to adverse effects:

Hepatotoxicity: Some coumarins, including coumarin itself, have been linked to hepatotoxicity (liver toxicity) when consumed in excessive amounts. Regulatory authorities in some countries

have established tolerable daily intake (TDI) levels for coumarin, prompting efforts to limit its presence in certain foods (77).

Photosensitivity: Specific coumarins, such as bergapten found in certain citrus fruits, can induce photosensitivity when applied topically or ingested in large quantities. This can result in skin reactions upon exposure to sunlight, including sunburn and blistering (78). Similarly, psoralen can trigger photosensitization reactions when the skin encounters ultraviolet (UV) light, leading to skin irritation, rashes, and an elevated risk of sunburn (79).

Anticoagulant Risk: Certain coumarin derivatives, like warfarin, serve as anticoagulant medications to prevent blood clotting. While effective for their intended purposes, these medications require vigilant monitoring and precise dosing due to their narrow therapeutic window. Overdosing can result in bleeding complications, underscoring the importance of medical supervision (80).

In conclusion, coumarins encompass a wide range of biological activities, but their safety and toxicity profile hinge on various factors, including the specific coumarin compound, dosage, and individual susceptibility. While natural dietary intake is generally safe, vigilance is essential when dealing with coumarin derivatives used in pharmaceuticals and high-concentration cosmetic products. Regulatory guidelines and risk management strategies are vital to ensure the safe utilization of coumarins across various applications.

7. CONCLUSION

Coumarins, with their intriguing benzopyrone structure, have long captivated the attention of researchers and enthusiasts alike due to their widespread presence in nature and versatile pharmacological properties. The plethora of biological activities exhibited by coumarins, including antithrombotic, anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer, and neuroprotective effects, highlights their vast potential in both pharmaceutical and nutraceutical applications. Moreover, the employment of efficient extraction methods, such as maceration, microwave-assisted extraction, and supercritical fluid extraction, enables the retrieval of these compounds for further investigation and industrial exploitation. However, it is crucial to acknowledge the potential health risks associated with excessive consumption of coumarin-rich substances, including hepatotoxicity, photosensitivity, and anticoagulant effects. Therefore, careful consideration must be given to the safe usage of coumarins, involving thorough risk assessments, stringent quality control measures, and adherence to established regulations. Despite these caveats, the study of coumarins continues to captivate scientists, offering a wealth of opportunities for groundbreaking discoveries and innovations in medicine, food science, and related disciplines. By responsibly harnessing the power of coumarins, we may unlock novel therapeutic pathways and create new possibilities for improving human health and well-being.

8. CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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