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A REVIEW ON THE STRUCTURE, DISTRIBUTION, AND BIOLOGICAL ACTIVITIES OF BIFLAVONOIDS IN CLUSIACEAE

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ABSTRACT
Background: The Clusiaceae family is widely distributed in tropical and subtropical climates, with a rich source of structurally diverse metabolites. Among these, biflavonoids stand out due to their complex structures and significant pharmacological activities. This review aims to examine data on various Clusiacean biflavonoids, their structural diversity, and bioactivity, thereby increasing the understanding of their implications in both traditional and modern medicine. **Methodology:** Data were collected from electronic databases like PubMed, Elsevier, Springer, and Google Scholar. The structural categorization of biflavonoids was based on the nature of linkage and substituent variations that influence their biological activities. Their distribution across Clusiacean members further highlights their chemotaxonomic importance. Additionally, structure-activity relationship studies reveal that specific linkages and functional groups enhance biological activity. **Results and Discussion:** Studies indicate that Clusiacean biflavonoids are potential candidates for drug discovery and therapeutic development due to their varied pharmacological activities. Approximately 21 biflavonoids from Clusiacean members are included in this review; nine of them exhibit antioxidant, five antimicrobial, five early antigen inhibition, four monoamine oxidase inhibition, three anti-inflammatory, three neuromuscular transmission inhibition, one antidiabetic, one anti-aging, and one hypocholesterolemic activity. **Conclusion:** Clusiacean biflavonoids possess desirable compounds for drug development owing to their diverse pharmacological activities, including antiviral, anti-inflammatory, anti-cancer, antioxidant, and neuroprotective properties, and so on. Overall, this article analyzes different biflavonoids obtained from Clusiaceae species, focusing on their structural diversity, biological importance, and structure-activity relationships.

INTRODUCTION

The Clusiaceae family comprises approximately 14 genera and nearly 800 species, with a wide distribution in the tropics. The family is distinguished by the presence of secondary metabolites, including xanthones, benzophenones, flavonoids, coumarins, terpenoids, and other compounds important in plant

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science and potential biological activity studies [1]. The plants in this family are traditionally used to treat ailments such as inflammation, infections, and skin diseases [2]. They are also economically valued for their fruits, resins, dyes, essential oils, and timber [3]. Biflavonoids are polyphenolic compounds composed of two identical or non-identical flavone-flavonoid units connected symmetrically or asymmetrically. Additionally, they are the dimers of the subunits flavone–flavone, flavone–flavanone, and flavanone–flavanone. These units can be connected through a carbon-carbon or a carbon-linear fragment-carbon linkage. Such specific arrangements and linkages determine their structural diversity and subtypes of bioflavonoids [4].

The majority of 592 biflavonoids are found in angiosperms, although they are also found in ferns, gymnosperms, and bryophytes [4]. Their potential health benefits are supported by frequent reports of their presence in plants used in both traditional and modern medicine. Therefore, they have become the focus of the scientific community in recent years, as they possess several pharmacological activities, including antibacterial, antiviral, antidiabetic, antioxidant, anti-inflammatory, antitumor, and cytotoxic properties. This makes them a potential component of several therapeutics, primarily for the treatment of diseases such as cardiovascular diseases, cancer, and neurological disorders [5]. Due to their chemical and biological significance, various bioprospective phytochemical research and chemical techniques utilizing coupling and molecular rearrangement strategies have been developed to identify and synthesize novel bioactive biflavonoids [6]. Although several biflavonoids have been identified from Clusiaceae, many remain structurally unexplored. Advanced techniques, such as 2D NMR and crystallography, are necessary to confirm their complex structures [4].

The existing literature primarily targets one or more specific species or genera within the family. Pharmacological studies are mainly limited to antioxidant activity, with fewer investigations into anti-inflammatory, antimicrobial, neuroprotective, or enzyme-inhibitory effects. Additionally, the mechanistic basis of activity and structure-activity relationships remains poorly understood. This review aims to examine data on various Clusiacean biflavonoids, their structural diversity, bioactivity, and structure-activity relationships, thereby increasing our understanding of their implications in both traditional and modern medicine.

MATERIAL AND METHODS

Relevant literature on biflavonoids from the family Clusiaceae was systematically collected using electronic databases, including PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect. Search strings combined keywords and Boolean operators such as “Biflavonoids and Clusiaceae”, “Biflavonoids and Bioactivities”, and specific compound names like Amentoflavone and Morelloflavone were queried. Studies were included if they reported the isolation, structure, distribution, or biological activities. Publications not specific to biflavonoids of Clusiaceae, non-English articles, and abstracts or theses without complete data are excluded. Titles and abstracts were screened, followed by full-text evaluation. Data on compound name, distribution, and biological activities were extracted. The findings were organized into four major areas: structural classification, distribution, biological activities, and structure-activity relationships of biflavonoids in the Clusiaceae family. Results were summarized and supported with tables and figures to highlight trends and research progress.

RESULTS AND DISCUSSION

Structural Classification of Biflavonoids

Biflavonoids are a unique category of polyphenolic compounds produced by the dimerization of two flavonoid units. They are structurally varied based on the nature and position of the linking structures that link each of the flavonoid units [7]. Biflavonoids are classified based on their interflavonoid bonding, i.e., C-C, C-O-C ether bridges, and multiple C-C bonds that control their structural constraints and bioactivity. The dimerization of the apigenin unit is a pattern commonly found in biflavonoids. There are several conceivable pairings. For instance, when two apigenin motifs are linked together using an 8 → 3′ connector, Amentoflavone is produced. Similarly, when two apigenin motifs are linked together using an 8 → 6 linker, Agathisflavone is produced. Additional combinations are shown in Figure 1a. As seen in Figure 1b, dimerization can also occur through an ether bond, utilizing one of apigenin's hydroxyl groups. This is true for substances like Ochnaflavone, Hinokiflavone, and a few other biflavonoids of the C-O-C type [8]. They are also classified based on flavonoid subclasses, e.g., flavone-flavone, flavanone-flavone, and flavanone–flavanone. Among the major biflavonoids identified in Clusiaceae, Amentoflavone (Figure 2) is one of the most abundant, consisting of two apigenin (flavone) units linked at C-8 and C′-3′. It is considered a structural prototype for many related compounds [9]. Morelloflavone

(Figure 3), a well-studied flavone–flavanone dimer, is commonly reported from *Garcinia* species, with the linkage occurring between C-3′ of the flavanone and C-8 of the flavone [10]. In addition, GB-1 (Figure 5) & GB-2 (Figure 6) are flavanone–flavanone dimers characteristic of *Garcinia*, differing in their C–C linkages, with GB-1 linked at C-3′–C-8″ & GB-2 at C-3′–C-6″ [11,12]. Another essential biflavonoid complex is Kolaviron (Figure 4), a mixture of closely related biflavonoids (including GB-1, GB-2 & Kolaflavanone) primarily isolated from *Garcinia kola*. These molecules belong to the flavanone–flavanone type of biflavonoids and are characterized by carbon–carbon linkages between the two flavanone units [13].

Distribution of Biflavonoids in Clusiaceae

Biflavonoids are widely distributed in the members of the Clusiaceae family, such as *Garcinia*, *Clusia*, and *Calophyllum*. The concentration and types of biflavonoids can also vary significantly between species and even within different parts of the same plant, including bark, seeds, leaves, and resin, where they contribute to the plant's defense mechanisms [15].

Chemotaxonomic relevance of Biflavonoid distribution in Clusiaceae: Their distribution patterns within the family are considered to be chemotaxonomically relevant, and also aid in the family's categorization and evolutionary research [16]. The chemotaxonomic relevance of biflavonoid distribution in Clusiaceae is provided in Table 1. Additional chemotaxonomic clarity is revealed when comparing at the family level. Biflavonoids are scarce or nearly absent in *Calophyllaceae* and *Hypericaceae*, whereas they are varied in Clusiaceae. Therefore, the dominance of biflavonoids in Clusiacean members provides evidence of phylogenetic independence from related lineages, as well as taxonomic delimitation [19], [20]. The distribution of biflavonoids within the tribes in Clusiaceae provides critical evolutionary and taxonomic insights. A shared ancestral capacity for 3′–8″ coupling enzymes is suggested by the dominance of 3′–8″ linked Biflavones in Garcinieae, which supports the monophyly of the tribe's chemical phenotype and helps to distinguish it from other clusioid lineages where morphology may be unclear [7]. An analogous retention and diversification of metabolic pathways can be seen in Clusieae, where biflavonoid biosynthesis was maintained but further developed with highly prenylated benzophenones. This suggests potential ecological adaptations, such as improved defense in reproductive tissues, in addition to supporting tribe delimitation [17]. Biflavonoids, xanthones, and benzophenones are consistently found together in Symphonieae, which reinforces tribal solidarity and suggests that these metabolites were originally present in a common ancestor and retained during diversification. The monophyly of Symphonieae is supported by this conserved chemical profile, which also aids in cross-validating molecular phylogenies [18]. The distribution of biflavonoids among these three tribes as a whole demonstrates their usefulness as chemotaxonomic identifiers. It reveals how conserved biosynthetic features and lineage-specific elaborations provide insight into the evolutionary history of Clusiaceae.

Biflavonoids in Clusiaceae

Various biflavonoids obtained from different members of the Clusiaceae family, along with their bioactivities, are provided in Table 2.

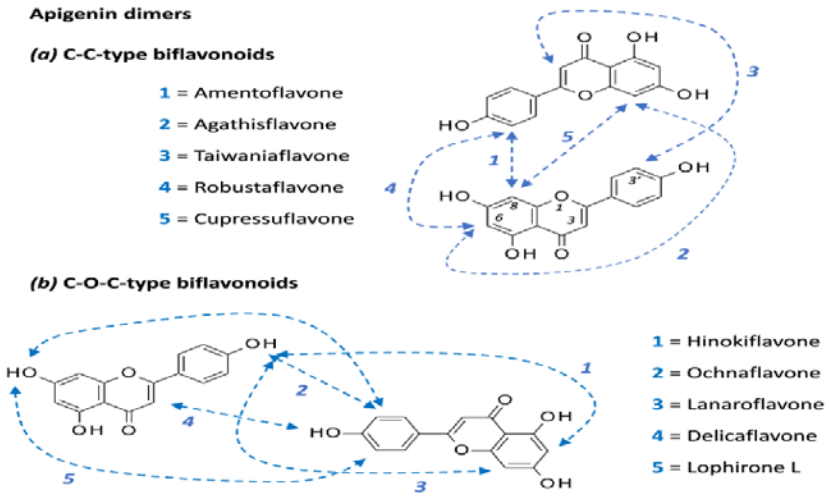

Figure 1: Formation of C-C type biflavonoids (a) and C-O-C type biflavonoids (b) with examples [8].

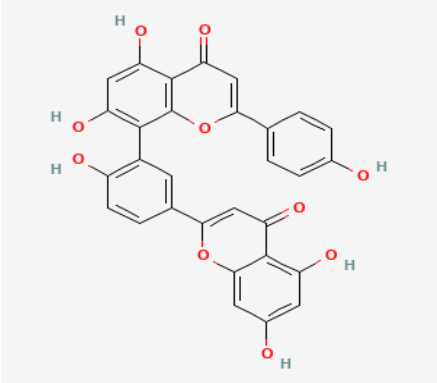

Figure 2: Amentoflavone [9]

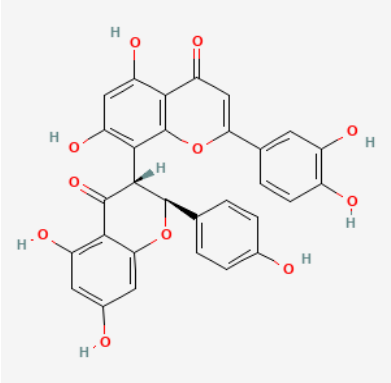

Figure 3: Morelloflavone [10]

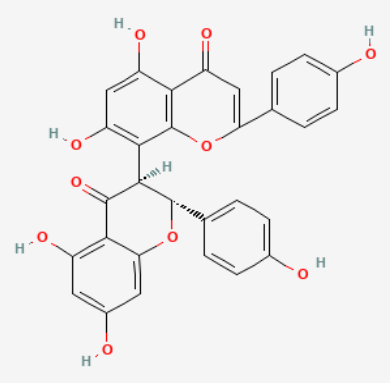

Figure 4: Kolaviron [14]

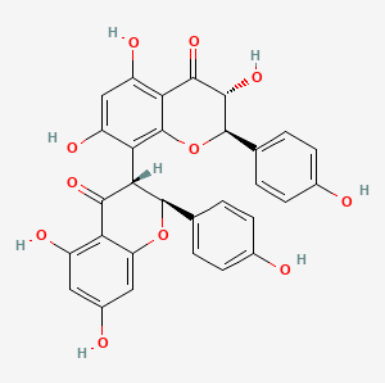

Figure 5: GB 1 [11]

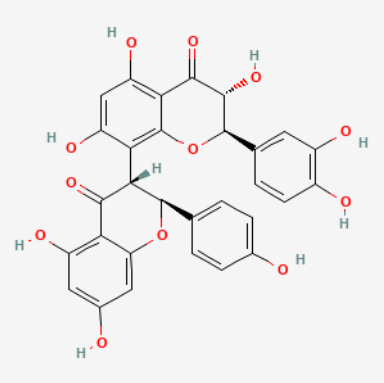

Figure 6: GB 2 [12]

Table 1: Chemotaxonomic relevance of Biflavonoid distribution in Clusiaceae

Taxonomic Group	Major Biflavonoid Types/Linkages	Representative Compounds	Chemotaxonomic Significance	Ref
Tribe Garcinieae (*Garcinia*, *Pentadesma*)	3’–8’’ Biflavones (flavone – flavone, flavone–flavanone)	Amentoflavone Morelloflavone GB 2a	Consistent occurrence acts as a diagnostic marker for Garcinieae	[7]
Tribe Clusieae (*Clusia*)	3’–8’’ Biflavones (flavone–flavone)	Amentoflavone derivatives	Occur with prenylated benzophenones and forms a chemical fingerprint for Clusieae	[17]
Tribe Symphonieae (*Symphonia*)	3’–8’’ Biflavones (flavanone–flavone variations)	Amentoflavone	Occur alongside xanthones and benzophenones and the presence across Symphonieae chemotaxonomically support tribal cohesion	[18]

Table 2: Biflavonoids obtained from different members of Clusiaceae along with their bioactivities

Biflavonoid	Source Plant	Bioactivity	Study Design and Sample Size	Limitations	Ref
Morelloflavone	*Garcinia cowa*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay, concentrations in triplicates.	No *in vivo* validations, Only the activity of root bark is studied.	[21]
	Garcinia dulcis	Hypocholesterolemic activity	*In vitro*, enzyme kinetic assay against HMG-CoA reductase, concentrations in triplicates.	No *in vivo* validations, only enzymatic evidence was presented for inhibition.	[22], [23]
	Garcinia volkensis	Anti-aging activity	*In vitro*, protein Glycation/AGE assay,	Limited biological replication of plant material, no *in vivo*	[24]

Biflavonoid	Source Plant	Bioactivity	Study Design and Sample Size	Limitations	Ref
			3 biological replicates used.	validations, not reported number of replicates.	
	Garcinia gardneriana	Monoamine Oxidase Inhibitory activity	*In vitro*, MAO enzyme assay, 4-6 concentrations in triplicates.	No *in vivo* validations, only branch of the plant was analysed, possible assay interference from polyphenols.	[25]
	Garcinia griffithii	Antioxidant activity	*In vitro*, DPPH radical scavenging assay.	No *in vivo* validations, no information on how many biological replicates used, not mentioned technical replicates for bioassay.	[26], [27]
	Calophyllum panciflorum	Inhibits EBV early antigen activation	*In vitro*, EBV early antigen activation assay in Raji cells.	No *in vivo* validations, only stem barks of the plant was analysed, number of replicates and error bars are not included, lacks comparative potency of known biflavonoids vs. new one.	[28]
Morelloflavone-7''-O-glucoside	*Garcinia griffithii*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay.	No *in vivo* validations, no information on how many biological replicates used, not mentioned technical replicates for bioassay.	[26], [27]
Morelloflavone-4‴-O-β-d-glycoside	*Garcinia brasiliensis*	Antioxidant activity	In vitro, DPPH scavenging assay, conducted in triplicates.	No *in vivo* validations, small number of phenolic compounds isolated and tested, study limited to epicarp.	[29]
Volkensiflavone	*Garcinia gardneriana*	Monoamine Oxidase Inhibitory activity	*In vitro*, MAO enzyme assay, 4-6 concentrations in triplicates.	No *in vivo* validations, only branch of the plant was analysed, possible assay interference from polyphenols.	[25]
Amentoflavone	*Garcinia brasiliensis*	Anti-inflammatory activity	*In vitro*, Neutrophil oxidative burst analysed through WST-1 reduction and luminol-enhanced chemiluminescence, Neutrophils and erythrocytes were obtained from healthy human donors.	No *in vivo* validations, did not explore pharmacokinetics, metabolism, or potential toxicity of the biflavonoids, donor-to-donor variability in neutrophil and erythrocyte responses not addressed.	[30]
	Calophyllum panciflorum	Inhibits EBV early antigen activation	*In vitro*, EBV early antigen activation assay in Raji cells.	No *in vivo* validations, only stem barks of the plant was analysed, number of replicates and error bars are not included, lacks comparative potency of known biflavonoids vs. new one.	[28]
Amento-4''-methylether	*Garcinia griffithii*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay.	No *in vivo* validations, no information on how many biological replicates used, not mentioned technical replicates for bioassay.	[26], [27]

Biflavonoid	Source Plant	Bioactivity	Study Design and Sample Size	Limitations	Ref
Fukugiside	*Garcinia cowa*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay, concentrations in triplicates.	No in vivo validations, only the activity of root and bark is studied.	[21]
	Garcinia brasiliensis	Antioxidant activity	In vitro, DPPH scavenging assay, conducted in triplicates.	No *in vivo* validations, small number of phenolic compounds isolated and tested, study limited to epicarp.	[29]
Fukugetin	*Garcinia brasiliensis*	Anti-inflammatory activity	*In vitro*, Neutrophil oxidative burst analysed through WST-1 reduction and luminol-enhanced chemiluminescence, Neutrophils and erythrocytes were obtained from healthy human donors.	No *in vivo* validations, did not explore pharmacokinetics, metabolism, or potential toxicity of the biflavonoids, donor-to-donor variability in neutrophil and erythrocyte responses not addressed.	[30]
Macrophylloflavone	*Garcinia macrophylla*	Antibacterial activity Antioxidant activity Antidiabetic activity	*In vitro*, antibacterial assay against *E. coli* and S. aureus, DPPH radical scavenging assay; *In vivo*, anti-type 2 diabetes mellitus activity in rats; mean ± SD in triplicates.	Unclear animal group size, Dose range is very narrow with 6-8 µg/kg body weight, only two bacterial strains were tested.	[31]
3,8''-Binaringenin	*Garcinia griffithii*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay.	No *in vivo* validations, no information on how many biological replicates used, not mentioned technical replicates for bioassay.	[26], [27]
	Pentadesma grandifolia	Antifungal activity	*In vitro*, antifungal assay via bio autography against *Cladosporium*, stem bark, roots, leaves and fruits of the plant were analysed.	No *in vivo* validations, incomplete reporting of replicates, bioautography is a qualitative/semi-quantitative method, without MIC the practical efficacy is unclear.	[32]
3,8''-Binaringenin-7''-O-glucoside	*Garcinia griffithii*	Antioxidant activity	*In vitro*, DPPH radical scavenging assay.	No *in vivo* validations, no information on how many biological replicates used, not mentioned technical replicates for bioassay.	[26], [27]
3,6” - Binaringenin	*Pentadesma grandifolia*	Antifungal activity	*In vitro*, antifungal assay via bio autography against *Cladosporium*, stem bark, roots, leaves and fruits of the plant were analysed.	No *in vivo* validations, incomplete reporting of replicates, bioautography is a qualitative/semi-quantitative method, without MIC the practical efficacy is unclear.	[32]
GB-1	*Calophyllum panciflorum*	Inhibits EBV early antigen activation	*In vitro*, EBV early antigen activation assay in Raji cells.	No *in vivo* validations, only stem barks of the plant was analysed, number of replicates	[28]

Biflavonoid	Source Plant	Bioactivity	Study Design and Sample Size	Limitations	Ref
				and error bars are not included, lacks comparative potency of known biflavonoids vs. new one.	
	Garcinia kola	Antibacterial activity	*In vitro*, standard antimicrobial assays against *S. mutans*, biological replicate at the microorganism level, multiple concentrations were tested and replicates.	No *in vivo* or clinical testing to verify oral efficacy, safety, or pharmacokinetics, limited range of organisms, for use as chewing stick the compound's solubility and stability is not addressed.	[33], [34]
GB 2	*Calophyllum panciflorum*	Inhibits EBV early antigen activation	*In vitro*, EBV early antigen activation assay in Raji cells.	No *in vivo* validations, only stem barks of the plant was analysed, number of replicates and error bars are not included, lacks comparative potency of known biflavonoids vs. new one.	[28]
	Garcinia buchananii	Decreases inhibitory neuromuscular transmission	*In vitro*/ *ex vivo*, Pellet propulsion assays, microelectrode readings, Receptor functional assays; Porcine ileum and descending colon from n = 4-7 animals and guinea pig colon from n = 3-4 in various fractions.	Possible differences can occur in complex *in vivo* environment, Possible species-differences by using porcine and guinea pig tissues, GBB inhibited propulsive motility in guinea pig colon, the isolated compounds did not replicate that effect in the pellet propulsion assay.	[35]
GB-2a	*Garcinia gardneriana*	Monoamine Oxidase Inhibitory activity	*In vitro*, MAO enzyme assay, 4-6 concentrations in triplicates.	No *in vivo* validations, only branch of the plant was analysed, possible assay interference from polyphenols.	[25]
GB-2a-7-O-glucoside	*Garcinia gardneriana*	Monoamine Oxidase Inhibitory activity	*In vitro*, MAO enzyme assay, 4-6 concentrations in triplicates.	No *in vivo* validations, only branch of the plant was analysed, possible assay interference from polyphenols.	[25]
Manniflavanone	*Garcinia buchananii*	Decreases inhibitory neuromuscular transmission	*In vitro*/ *ex vivo*, Pellet propulsion assays, microelectrode readings, Receptor functional assays; Porcine ileum and descending colon from n = 4-7 animals and guinea pig colon from n = 3-4 in various fractions.	Possible differences can occur in complex *in vivo* environment, Possible species-differences by using porcine and guinea pig tissues, GBB inhibited propulsive motility in guinea pig colon, the isolated compounds did not replicate that effect in the pellet propulsion assay.	[35]
Buchananiflavonone	*Garcinia buchananii*	Decreases inhibitory neuromuscular transmission	*In vitro*/ *ex vivo*, Pellet propulsion assays, microelectrode readings, Receptor functional assays; Porcine ileum and	Possible differences can occur in complex *in vivo* environment, Possible species-differences by using porcine and guinea pig tissues, GBB inhibited propulsive motility	[35]

Biflavonoid	Source Plant	Bioactivity	Study Design and Sample Size	Limitations	Ref
			descending colon from n = 4-7 animals and guinea pig colon from n = 3-4 in various fractions.	in guinea pig colon, the isolated compounds did not replicate that effect in the pellet propulsion assay.	
Podocarpusflavone	*Garcinia brasiliensis*	Anti-inflammatory activity	*In vitro*, Neutrophil oxidative burst analysed through WST-1 reduction and luminol-enhanced chemiluminescence, Neutrophils and erythrocytes were obtained from healthy human donors.	No *in vivo* validations, did not explore pharmacokinetics, metabolism, or potential toxicity of the biflavonoids, donor-to-donor variability in neutrophil and erythrocyte responses not addressed.	[30]
Kolaviron	*Garcinia kola*	Antioxidant, Hematological modulation, Lipid-modulating and hepatoprotective activities.	*In vivo*, hematological, biochemical, hormonal, oxidative stress, and histopathological analysis, 56 Wistar rats (male and female) were used.	The highest dose is 500 mg/kg, but possible effects in even higher ranges 90 days were not assessed, reversibility was studied only for 30 days, the duration may not be sufficient for certain longer-term effects or for tissues with slower turnover.	[36]
Dulcisbiflavonoid B and C	*Garcinia dulcis*	Antimicrobial activity	*In vitro*, antimicrobial activity against various microorganisms.	No *in vivo* validations, the exact number of samples used for the extraction and isolation process is not specified, antimicrobial activities of some isolated compounds were evaluated, but the study does not specify the range of microorganisms tested or the results in detail.	[37]
	Calophyllum panciflorum	Inhibits EBV early antigen activation	*In vitro*, EBV early antigen activation assay in Raji cells.	No *in vivo* validations, only stem barks of the plant were analyzed, the number of replicates and error bars are not included, lacks comparative potency of known biflavonoids vs. the new one.	[28]

BIOACTIVITY OF BIFLAVONOIDS IN CLUSIACEAE

Biflavonoids produced from numerous members of the Clusiaceae family have a diverse variety of biological functions, including antioxidant, anti-diabetic, anti-inflammatory, anti-bacterial activity, and so on.

Antioxidant activity

Among the biflavonoids identified, 9 of them showed antioxidant activity. Morelloflavone and Fukugiside, identified from *G. cowa*, showed elevated antioxidant properties. The stem and leaves of *G. cowa* were extracted using methanol, and the resulting extract yielded biflavonoids, including Volkensiflavone, Morelloflavone, and Fukugiside. The antioxidant activity of the extracts, as well as that of isolated components, was evaluated using a DPPH radical scavenging assay. The isolated Morelloflavone and Fukugiside showed high activity with IC_{50} values of 10.01 and 12.92 μg/mL, respectively [21]. The biflavonoids, such as Fukugetin, Amentoflavone, and

Podocarpusflavone, derived from G. brasiliensis, constitute a therapeutic approach for treating disorders associated with oxidative stress, reducing inflammation, and mitigating the damaging effects of ROS. Potent inhibitory effects were demonstrated on the oxidative burst of human neutrophils, inhibiting ROS production by 50% at a concentration of 1 μM L^{-1} [30]. Extraction and purification of leaf and stem bark extracts of *G. griffithii* yielded 5-biflavonoids: 3,8"-binaringenin, 3,8"-binaringenin-7"-O-glucoside, Amento-4"-methylether, Morelloflavone-7"-O-glucoside & Morelloflavone, which were spectroscopically characterized. Having an IC_{50} value of 96.4 ± 2.6 μg/mL, the n-hexane extract of the stem barks showed the highest radical scavenging activity in the antioxidant assay on DPPH radical scavenging [26], [27].

Anti-inflammatory activity

Compounds such as Amentoflavone, Podocarpusflavone and Fukugetin showed anti-inflammatory activity. The ethanolic extract of G. brasiliensis leaves showed anti-inflammatory and anti-nociceptive activity in rats and mice, supporting the traditional use of *Garcinia* species in treating urinary tract inflammation and inflammatory pain conditions, such as arthrosis.

Procyanidin, Fukugetin, Amentoflavone, as well as Podocarpus flavone A, discovered in G. brasiliensis, constitute therapeutic methods for oxidative stress-related disorders, limiting inflammation and lowering the damaging effects of ROSs. Procyanidins have been shown to reduce oxidative hemolysis with 88±7% inhibition at 50 μmol L^{-1} and lipidic peroxidation in human erythrocytes with malondialdehyde level of 8.5±0.3nmol/mg Hb at 50 μmol L^{-1}, confirming anti-inflammatory as well as antioxidant effects of *G. brasiliensis* [30].

Anti-hyperglycaemic and Antidiabetic Activity

Macrophylloflavone was effectively extracted from the ethyl acetate fraction of the stem bark of the *G. macrophylla* plant. Flavonoids can help prevent diabetes by stimulating the regeneration of β-cells in the pancreas, which are responsible for producing insulin. Formation turns glucose into energy, allowing it to decline, hence keeping a normal level of blood glucose. The variants demonstrated superior antidiabetic action compared to the positive control. However, a dose of 6μg per kg body weight significantly decreased the level of blood glucose in diabetic rats [31].

Antibacterial Activity

Two of the identified compounds, Macrophylloflavone and GBI, showed antibacterial activity. According to the findings, the antimicrobial strength is classified as mild, moderate, or firm based on inhibition zones (<12, 12-20, and >20 mm). The isolate from the ethyl acetate extract was characterized with spectroscopic data of *G. macrophylla*. Macrophyllo-flavone demonstrated high antibacterial action against *E. coli* and *S. aureus* bacteria. Inhibition zones for E. coli at different concentrations ranged from 16.65 ± 0.43 to 20.29 ± 0.28 mm & for *S. aureus*, inhibition zones ranged from 15.54 ± 0.39 to 23.16 ± 0.32 mm [31]. The antibacterial component was separated from the active fraction using silica gel chromatography. NMR analysis was used to identify the purified molecule. Standard microbiological tests were used to evaluate the antibacterial properties of the purified compound. GB 1 was determined from the ether fraction of *G. kola*, which exhibited antibacterial activity with an MIC ranging from 32 to 64 μg/ml. GB1 demonstrated efficacy against *S. mutans*. GB1 caused *S. mutans* to aggregate and showed moderate bactericidal effect against the bacteria at MIC 256μg/ml [33], [34].

Antimicrobial and Antifungal Activity

Compounds such as Dulcisbiflavonoid B showed antimicrobial activity, and C, 3,8″-Binaringenin, and 3,6″-Binaringenin showed antifungal activity. Stem extract of *G. dulcis* has been investigated, and two biflavonoids, Dulcisbiflavonoid B as well as Dulcisbiflavonoid C, have been isolated. Isolates' structures were determined via spectroscopic analysis, and the results were compared to prior reports. The antimicrobial properties of various isolated substances were investigated [37]. *P. grandifolia* stem bark, roots, leaves, and fruits were tested for xanthones, biflavonoids, and triterpenoids. Structures were determined by NMR and mass spectrometry, as well as by 13C-NMR CSEARCH and SPECINFO database systems. Two biflavonoids, 3,8″-Binaringenin and 3,6″-Binaringenin, have been identified along with other compounds. 3,8″-Binaringenin and 3,6″-Binaringenin exhibit antifungal efficacy against *Cladosporium sphaerospermum* [32].

Monoamine Oxidase (MAO) Inhibitory Activity

Compounds such as Morelloflavone, Volkensiflavone, GB 2a, and its derivatives showed MAO inhibitory action. The study described the chemical makeup of *G. gardneriana* branch extract to measure the MAO inhibitory activity of separated

biflavonoids. Morelloflavone, Volkensiflavone, Gb-2a, as well as Gb-2a-7-O-glucoside, were identified through spectroscopic and spectrometric data in the ethyl acetate fraction of the ethanol extract of the branch. Compounds reduced MAO-A activity in vitro, with IC_{50} values varying between 5.05-10.7 μM and 20.7-66.2 μM for MAO-B [25].

Antitumor and Anticancer Activity

Amentoflavone, Morelloflavone, GB1, and GB2 were isolated and identified through spectroscopic and spectrometric data from the EtOH extract of the stem bark of *C. panciflorum*. It showed inhibitory activity against induced EBV early antigen activation, which acts as a potential antitumor-promoting pathway [28].

Hypolipidemic and Hypocholesterolemic Activity

Morelloflavone obtained from *G. dulcis* was tested for its impact on HMG-CoA reductase, a rate-limiting enzyme in the cholesterol biosynthesis pathway. The house mouse HMG-CoA reductase's catalytic domain was used to demonstrate that Morelloflavone inhibited the enzyme's activity by competing with HMG-CoA, while being non-competitive with NADPH, with inhibition constants of 80.87 ± 0.06 μM. Naringenin also inhibited HMG-CoA at 83.58 ± 4.37 μm, while luteolin had an inhibition constant of 83.59 ± 0.94 μm. Not all of the compounds were competitive with NADPH [22], [23].

Anti-aging Activity

Morelloflavone obtained from *G. volkensis* shows anti-aging activity. High-performance liquid chromatography (HPLC) and normal phase column chromatography were used to isolate the phenolic components. The study aimed to identify compounds that inhibit Advanced Glycation End-products. Polyphenolic compounds with phloroglucinol moieties, such as Morelloflavone, were extracted and identified from *G. volkensis*. The results further indicated that Morelloflavone is an excellent inhibitor of AGE formation, exhibiting IC_{50} values of 78 and 64 μM [24].

Insights on Structure-Activity Relationship

The members of the Clusiaceae family contain a large number of biflavonoids with several bioactivities like anti-inflammatory, antioxidant, antiviral, anticancer, and antibacterial actions [38].

Understanding the Structure-Activity Relationship of each compound helps us optimize their medicinal applications and further therapeutic developments [39].

Role of Linkages in Bioactivity

Clusiacean biflavonoids are predominantly made up of C-C linkages, which are commonly linked with high antioxidant and anticancer activities. Compounds like Amentoflavone inhibited the proliferation of SiHa and CaSki cells with GI_{50} values of 47.2±9.64 μM and 29.1±4.38 μM, respectively [40]. Radical-scavenging assays suggested that Amentoflavone effectively scavenge •O2 -, DPPH•, ABTS• radicals with IC_{50} values 8.98±0.23, 432.25±84.05, 7.25±0.35 μM respectively [41]. The C-O-C connections can modify the bioavailability and solubility, which tend to enhance its anticancer and neuroprotective activities. In compounds like Hinokiflavone, with C-O-C linkages, these linkages have shown modest cytotoxic potential against various cancer cell lines, with IC_{50} values of 19.0, 29.8, and 39.3 μg/ml against HeLa (cervix), U251 (glioma), and MCF7 (breast) cancer cells, respectively [42]. Compounds like Kolaviron, with mixed linkages, exhibit elevated antioxidant activity, as indicated by an IC value of 82.87±9.26 μM. It also shows anti-inflammatory activity with concentrations 20.48±5.13, 231.26 ±78.31, and 9.49±2.5pg/mL against IL-1β, MCP-1, and VEGF, respectively [43]. The role of different linkages, their structural traits, and associated bioactivities are provided in Table 3.

Table 3: Role of Linkages in Bioactivity of Biflavonoids

Linkage Type	Example Compounds	Structural Traits	Associated Bioactivities	Ref
C–C (3′–8″)	Amentoflavone, Agathisflavone, Ginkgetin	Highly rigid, planar dimer, strong π–π stacking potential	Potent **antioxidant, anticancer,** antiviral and effective ROS scavenging activity	[7], [40], [41]
C–O–C (Ether-linked)	Hinokiflavone, Robustaflavone	Flexible ether bond introduces polarity and conformational adaptability	Improved **bioavailability** and **solubility,** anticancer, **antimicrobial, neuroprotective** and antiviral activities	[8], [42]
Mixed linkages (C–C + C–O–C)	Kolaviron (GB1, GB2, Kolaflavanone mixture)	Combination of rigid and flexible linkages with complex stereochemistry	**Antioxidant, antidiabetic,** hepatoprotective and anti-inflammatory activities	[13], [43]

Role of Substituents in Bioactivity

Hydroxylation at certain sites can enhance the antioxidant activity. Although these substituents can lower the permeability of the cells, they are known to improve bioactivity through multiple hydroxylation. The effects of the 6″-OH group of Isoquercitrin were compared with Quercitrin, and their ROS scavenging activity was analyzed. Isoquercitrin (IC_{50} 78.16 ± 4.83 μM) demonstrated greater ROS scavenging capacity than quercitrin (IC_{50} 87.99 ± 5.43 μM). Accordingly, the finding suggested that the addition of a 6'-OH group raises the antioxidant activity of flavonoid glucosides. [44]. Methoxylation is found to enhance membrane permeability and lipophilicity, which enhances antibacterial and anticancer activities. The anti-proliferative effects of a rare flavone, 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone, isolated from *Glycomis ovoidea*, were studied against MCF-7 breast cancer cells. By replacing the C4′-OH group with a methoxy group, the compound demonstrated stronger IC_{50} values of 3.71 μM [45]. Prenyl groups are also known to enhance membrane contact, lipophilicity, cytotoxicity, and antiviral activities. Experiments show that C8-prenylation of a flavonoid enhances cytotoxicity, inducing apoptotic cell death in H4IIE cells without affecting its antioxidative properties.

The prenylated compounds, such as Licoflavone C and Isobavachin, exerted an enhanced toxicity in both H4IIE (IC_{50} values of 42±5 and 96±19μmol/L) and C6 cells (IC_{50} values of 37±6 and 69±3μmol/L). At the same time, the non-prenylated analogs showed almost no cytotoxic effect [46]. The role of different substituents, their structural effects, and impact on bioactivities are provided in Table 4.

Table 4: Role of Substituents in Bioactivity of Biflavonoids

Substituents	Examples compounds	Structural Effect	Impact on Bioactivity	Ref
Hydroxyl groups (–OH)	Amentoflavone, Agathisflavone, Volkensiflavone	Increases polarity, enables hydrogen bonding and radical scavenging activity	Enhances **antioxidant activity** via H-donation and multiple hydroxylation boosts ROS neutralization, but may reduce **cell permeability**	[44]
Methoxy groups ($–OCH_3$)	Methoxylated Amentoflavone derivatives	Increases lipophilicity and membrane permeability and reduces polarity	Enhances **anticancer** and **antibacterial** activities via better cellular uptake, and excessive methoxylation lowers antioxidant potential due to reduced H-donation	[45]
Prenyl groups ($–C_5H_9$)	Guttiferones, Kolaviron	Strongly enhances lipophilicity and promotes interaction with lipid membranes	Improves **cytotoxic**, **antiviral**, and **anti-inflammatory** activities and facilitates better cell penetration and bioavailability	[46]

The type and position of their linkages and substituent groups have a high impact on the activity of biflavonoids. Structure-activity relationships optimize the properties of these compounds and enhance their therapeutic capabilities.

CONCLUSION

Clusiacean biflavonoids exhibit a broad range of pharmacological properties, including antiviral, anti-inflammatory, anticancer, antioxidant, and neuroprotective effects. Still, their utility is limited by poor solubility due to hydrophobicity, low intestinal permeability resulting from their high molecular weight, and extensive first-pass metabolism [47]. LC-MS/MS studies in rats revealed that Amentoflavone has extremely low oral bioavailability (0.04–0.06%), with 90–97% of the circulating metabolites being conjugated. While these retain antioxidant activity, their anticancer efficacy is diminished, explaining the gap between strong *in vitro* results and poor *in vivo* performance that has stalled clinical development [48]. Formulation strategies, such as nanoformulations, prodrugs, and co-crystallization, have been employed to address these limitations. Nano-formulations, which consist of liposomes and polymeric nanoparticles, can improve solubility and absorption. Soluplus–TPGS nanomicelles (~67 nm) encapsulating Amentoflavone showed high stability, enhanced cancer-cell cytotoxicity, and improved oral bioavailability in rats compared with Amentoflavone suspension [49]. Complex, multi-component nano platforms face limitations such as scalability, reproducibility, and CMC burdens, while novel substances require costly toxicology and regulatory reviews. Simple nanocrystals or micelles are more feasible for translation; however, no biflavonoid nanoformulation has entered clinical trials, unlike Curcumin or quercetin nanoformulations, which have already been tested *in vivo* [50]. The formation of prodrugs is said to improve permeability and metabolic stability. A smart nanoplatform combining Amentoflavone with doxorubicin, ferric ions, and

PEG-polyphenol achieved high water dispersion, extended circulation, pH-responsive release, modulation of the AKR1B10 and NF-κB pathways, and enhanced anticancer activity. The chemical difficulty of selectively altering many hydroxyl groups without sacrificing activity, the toxicological load of pro-moieties, and unpredictable enzymatic conversion resulting from variability are some of the main drawbacks. However, no valid biflavonoid prodrug has progressed past exploratory animal research, and before clinical translation is conceivable, thorough medicinal chemistry, early pharmacokinetics, and enzymology profiling will be required. [51].

Co-crystallization and solid dispersions are also a powerful means to improve dissolution and oral bioavailability. In the studies conducted, rats have been administered amorphous solid dispersions to enhance their systemic exposure, solubility, and dissolution rate. These formulation techniques have shown promising outcomes in preclinical cancer models, such as the inhibition of tumor growth in xenografted mice. Co-crystals and solid dispersions are scalable, economical, and have regulatory precedent; however, biflavonoids still face challenges such as low permeability, extensive first-pass metabolism, and stability issues. Despite high translational potential, no pure biflavonoid co-crystal has advanced beyond preclinical studies [47]. It emphasizes that although formulation-driven methods can improve bioavailability in experiments, they are still insufficient to overcome the inherent ADME obstacles that prevent clinical translation. Two recurrent patterns can be identified as the primary causes of translational defects of biflavonoids. First, even the most sophisticated oral delivery systems rarely produce clinically meaningful and long-lasting plasma levels of the parent chemical, a phenomenon known as exposure deficit. Since the majority of circulating conjugates produced by first-pass metabolism may not accurately mimic the in vitro potency or target profile of the parent molecule, a metabolite mismatch frequently occurs, resulting in decreased efficacy *in vivo* [52]. Despite broad bioactivities, biflavonoids often fail to show significant therapeutic effects due to low solubility, poor permeability, and rapid metabolism [47]. Research gaps persist in defining which biflavonoids warrant priority, elucidating their mechanisms of action, and establishing standardized methodologies for pharmacokinetic and toxicological evaluation. Compounds such as Amentoflavone, Ginkgetin, and Robustaflavone warrant deeper investigation given their broad bioactivities but persistent ADME challenges. Progress in overcoming these constraints will rely on the combination of robust, manufacturing-ready delivery systems that can provide clinically viable systemic exposure, along with medicinal chemistry techniques such as soft-prodrug design, masking phenolic groups, or scaffold cutting [52]. Despite their extensive bioactivity, Clusiaceae biflavonoids are nevertheless constrained by their low permeability, poor solubility, and quick metabolism. Certain preclinical developments show promise for formulation, including *Selaginella*-derived solid dispersions that inhibited tumor growth in mice and Amentoflavone nanomicelles with enhanced bioavailability [47]. However, persistent ADME obstacles are highlighted by failures such as the ~0.05% oral bioavailability of Amentoflavone and the loss of potency resulting from fast conjugation [48]. Early ADME and metabolite profiling, parallel medicinal chemistry and formulation optimization, *in vivo* pharmacokinetic and pharmacodynamics correlation, early safety de-risking, and scalable chemistry, manufacturing, and control development are all essential components of a well-defined roadmap. The transition of biflavonoids from encouraging preclinical results to clinical translation will require such an integrated approach.

ABBREVATIONS

GB1: *Garcinia* Biflavonoid 1; **GB2:** *Garcinia* Biflavonoid 2; **IC_{50}**: Inhibitory Concentration 50; **DPPH**: 2,2-Diphenyl-1-picrylhydrazyl; **EBV**: Epstein-Barr virus; **ROS**: Reactive Oxygen Species; **MIC**: Minimum Inhibitory Concentration; **MAO**: Monoamine oxidase; **HMG CoA**: 3-hydroxy-3-methylglutaryl coenzyme A; **NADPH**: Nicotinamide Adenine Dinucleotide Phosphate; **IL-1β**: Interleukin-1 beta; **MCP-1**: Monocyte Chemoattractant Protein-1; **VEGF**: Vascular Endothelial Growth Factor; **LC-MS/MS**: Liquid Chromatography-Tandem Mass Spectrometry; **TPGS**: d-α-tocopheryl polyethylene glycol 1000 succinate; **PEG:** Polyethylene Glycol; **ADME**: Absorption, Distribution, Metabolism and Excretion barriers.

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NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Anjana Unni took the lead in writing the manuscript and contributed to the conceptualization, study design, data

collection, literature review, and interpretation of results. Sheeja T Tharakan carried out the formal analysis and contributed to the interpretation of results, as well as to the refinement and finalization of the manuscript. All authors have made substantial, direct, and intellectual contributions to the work and have approved the final version for publication.

REFERENCES

[1] Kshirsagar P, Gaikwad S, Pai S, Desai N, Bapat V. Evaluation of antioxidant capacity and phytochemical investigation of eleven Clusiaceae members from Western Ghats, India. *Biocatalysis and Agricultural Biotechnology*, **44**, 102476 (2022) https://doi.org/10.1016/j.bcab.2022.102476.

[2] Diel KAP, Marinho LC, Von Poser GL. The ethnobotanical relevance of the tribe Symphonieae (Clusiaceae) around the world. *Journal of Ethnopharmacology*, **284**, 114745 (2022) https://doi.org/10.1016/j.jep.2021.114745.

[3] Raj SP, Solomon PR, Thangaraj B. *Biodiesel from flowering plants. Springer*, (2022) https://doi.org/10.1007/978-981-16-4775-8.

[4] He X, Yang F, Huang X. Proceedings of Chemistry, Pharmacology, Pharmacokinetics and Synthesis of Biflavonoids. *Molecules*, **26**, 6088 (2021) https://doi.org/10.3390/molecules26196088.

[5] Li H, Yang J, Wang M, Ma X, Peng X. Studies on the inhibition of α-glucosidase by biflavonoids and their interaction mechanisms. *Food Chemistry*, **420**, 136113 (2023) https://doi.org/10.1016/j.foodchem.2023.136113.

[6] Klischan MKT, Mazzone F, Berning L, Greb J, Schlamkow M, Haase M, Frey W, Stork B, Pfeffer K, Pietruszka J. Modular Approach for the Synthesis and Bioactivity Profiling of 8,8′-Biflavones. *ACS Omega*, **8**, 41816-34 (2023) https://doi.org/10.1021/acsomega.3c06503.

[7] Šamec D, Jurčević Šangut I, Karalija E, Šarkanj B, Zelić B, Šalić A. 3′-8″- Biflavones: A Review of Their Structural Diversity, Natural Occurrence, Role in Plants, Extraction and Identification. *Molecules*, **29**, 4634 (2024) https://doi.org/10.3390/molecules29194634.

[8] Goossens J-F, Goossens L, Bailly C. Hinokiflavone and Related C–O–C-Type Biflavonoids as Anti-cancer Compounds: Properties and Mechanism of Action. *Nat. Prod. Bioprospect.*, **11**, 365-77 (2021) https://doi.org/10.1007/s13659-021-00298-w.

[9] Wu S, Liu C, Li Y, Zhang X, Han Q, Zhao H, Zhao K, Dang Y, Wang R, Song S. Inhibitory mechanisms of Amentoflavone on amyloid-β peptide aggregation revealed by replica exchange molecular dynamics. *Sci Rep*, **15**, 24352 (2025) https://doi.org/10.1038/s41598-025-10623-9.

[10] Carrillo-Hormaza L, Ramírez AM, Osorio E. Chemometric classification of *Garcinia madruno* raw material: Impact of the regional origin and ripeness stage of a Neotropical exotic species. *Food Chemistry*, **293**, 291–8 (2019) https://doi.org/10.1016/j.foodchem.2019.04.118.

[11] Chen H-X, Yang F, He X-Q, Li T, Sun Y-Z, Song J-P, Huang X-A, Guo W-F. *Garcinia* Biflavonoid 1 Improves Lipid Metabolism in HepG2 Cells via Regulating PPARα. *Molecules*, **27**, 1978 (2022) https://doi.org/10.3390/molecules27061978.

[12] Hioki Y, Onwona-Agyeman S, Kakumu Y, Hattori H, Yamauchi K, Mitsunaga T. Garcinoic Acids and a Benzophenone Derivative from the Seeds of *Garcinia kola* and Their Antibacterial Activities against Oral Bacterial Pathogenic Organisms. *J. Nat. Prod.*, **83**, 2087–92 (2020) https://doi.org/10.1021/acs.jnatprod.9b01045.

[13] Fu T-D, Zhang W-L, Zhang Y, Deng C-S, Qi W, Xu Q, Song J-P, Guo W-F, Yang R-Y, Huang X-A. *Garcinia* Biflavonoid 1 from *Garcinia* kola Ameliorates Glycolipid Metabolism Disorder in Type 2 Diabetic db/db Mice. *Natural Product Communications*, **19**, 1934578X231224993 (2024) https://doi.org/10.1177/1934578X231224993.

[14] Alrazi IMD, Ogunwa TH, Kolawole AO, Elekofehinti OO, Omotuyi OI, Miyanishi T, Maruta S. Kolaflavanone, a biflavonoid derived from medicinal plant *Garcinia*, is an inhibitor of mitotic kinesin Eg5. *The Journal of Biochemistry*, **170**, 611–22 (2021) https://doi.org/10.1093/jb/mvab083

[15] Kshirsagar P, Gaikwad S, Pai S, Desai N, Bapat V. Evaluation of antioxidant capacity and phytochemical investigation of eleven Clusiaceae members from Western Ghats, India. *Biocatalysis and Agricultural Biotechnology*, **44**, 102476 (2022) https://doi.org/10.1016/j.bcab.2022.102476.

[16] Dai X-H, Zhu J-M, Wang G-Y, Ren Y-H, Liu H-L,Wang Y. Gymnosperm-specific CYP90Js enable biflavonoid biosynthesis and microbial production of Amentoflavone. *Nat Commun*, **16**, 7792 (2025) https://doi.org/10.1038/s41467-025-62990-6.

[17] Ferraz CG, Silva MDCC, Pereira DASG, Caldas BVV, Mattos R, Oliveira VVG, Andrade EMJ, Soares ACF, Da Silva F, Cruz FG, Ribeiro PR. Polyprenylated benzophenone derivatives from *Clusia burle-marxii* and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, **94**, 104218 (2021) https://doi.org/10.1016/j.bse.2020.104218.

[18] Téné D-G, Tih AE, Kamdem MHK, Talla RM, Diboue PHB, Melongo YKD, Dzukoug CR, Mmutlane EM, Ndinteh DT, Bodo B, Ghogomu RT. Antibacterial and antioxidant activities of compounds isolated from the leaves of *Symphonia globulifera* (Clusiaceae) and their chemophenetic significance. *Biochemical Systematics and Ecology*, **99**, 104345 (2021) https://doi.org/10.1016/j.bse.2021.104345.

[19] Ferdosh S. The Extraction of Bioactive Agents from *Calophyllum inophyllum* L., and Their Pharmacological Properties. *Sci. Pharm.*, **92**, 6 (2024) https://doi.org/10.3390/scipharm92010006.

[20] Rizzo P, Altschmied L, Ravindran BM, Rutten T, D'Auria JC. The Biochemical and Genetic Basis for the Biosynthesis of Bioactive Compounds in *Hypericum perforatum* L., One of the Largest Medicinal Crops in Europe. *Genes*, **11**, 1210 (2020) https://doi.org/10.3390/genes11101210.

[21] Phukhatmuen P, Raksat A, Laphookhieo S, Charoensup R, Duangyod T, Maneerat W. Bioassay guided isolation and identification of antidiabetic compounds from *Garcinia cowa* leaf extract. *Heliyon*, **6**, e03625 (2020) https://doi.org/10.1016/j.heliyon.2020.e03625.

[22] Siangcham T., Prathaphan P., Ruangtong J., Thongsepee N., Martviset P., Chantree P., Sornchuer P., and Sangpairoj K., Camboginol and Morelloflavone from *Garcinia dulcis* (Roxb.) Kurz flower extract promotes autophagic cell death against human glioblastoma cells through endoplasmic reticulum stress. *Prev Nutr Food Sci*., **27**, 376-383, (2022) https://doi.org/10.3746/pnf.2022.27.4.376.

[23] Tuansulong K, Hutadilok-Towatana N, Mahabusarakam W, Pinkaew D, Fujise K. Morelloflavone from *Garcinia dulcis* as a Novel Biflavonoid Inhibitor of HMG-CoA Reductase. *Phytotherapy Research*, **25**, 424–8 (2011) https://doi.org/10.1002/ptr.3286.

[24] Magadula J, Mbwambo Z, Gatto J, Derbré S, Guilet D, Richomme P. Polyphenolic Compounds with Anti-Ages Activity from Three Clusiaceae Plants. *EJMP*, **4**, 1336–44 (2014) https://doi.org/10.9734/EJMP/2014/10812.

[25] Recalde-Gil MA, Klein-Júnior LC, Dos Santos Passos C, Salton J, De Loreto Bordignon SA, Monace FD, Filho VC, Henriques AT. Monoamine Oxidase Inhibitory Activity of Biflavonoids from Branches of *Garcinia gardneriana* (Clusiaceae). *Natural Product Communications*, **12**, 1934578X1701200411 (2017) https://doi.org/10.1177/1934578X1701200411.

[26] Othman NA, Liew SY, Khaw KY, Ablat A, Karsani SA, Leong KH, Blanchard P, Derbré S, Awang K. Secondary metabolites from the barks of *Garcinia griffithii* T. Anderson (Clusiaceae) and their chemophenetic significance. *Biochemical Systematics and Ecology*, **116**, 104869 (2024) https://doi.org/10.1016/j.bse.2024.104869.

[27] Sazali NSAN, Ahmad F, Taher M, Salleh WMNHW. Biflavonoids from the leaves and stem bark of *Garcinia griffithii* and their biological activities. *mpj*, **21**, 889–97 (2017) https://doi.org/10.12991/mpj.2017.27.

[28] Ito C, Itoigawa M, Miyamoto Y, Rao KS, Takayasu J, Okuda Y, Mukainaka T, Tokuda H, Nishino H, Furukawa H. A New Biflavonoid from *Calophyllum panciflorum* with Antitumor-Promoting Activity. *J. Nat. Prod*., **62**, 1668–71 (1999) https://doi.org/10.1021/np990065j.

[29] Gontijo VS, De Souza TC, Rosa IA, Soares MG, Da Silva MA, Vilegas W, Viegas C, Dos Santos MH. Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. *Food Chemistry*, **132**, 1230–5 (2012) https://doi.org/10.1016/j.foodchem.2011.10.110.

[30] Saroni Arwa P, Zeraik ML, Farias Ximenes V, Da Fonseca LM, Da Silva Bolzani V, Siqueira Silva DH. Redox-active biflavonoids from *Garcinia brasiliensis* as inhibitors of neutrophil oxidative burst and human erythrocyte membrane damage. *Journal of Ethnopharmacology*, **174**, 410–8 (2015) https://doi.org/10.1016/j.jep.2015.08.041.

[31] Cane HPCA, Saidi N, Yahya M, Darusman D, Erlidawati E, Safrida S, Musman M. Macrophylloflavone: A New Biflavonoid from *Garcinia macrophylla* Mart. (Clusiaceae) for Antibacterial, Antioxidant, and Anti-Type 2 Diabetes Mellitus Activities. *The Scientific World Journal*, **2020**, 1-14 (2020) https://doi.org/10.1155/2020/2983129.

[32] Djoufack GLN, Valant-Vetschera KM, Schinnerl J, Brecker L, Lorbeer E, Robien W. Xanthones, Biflavanones and Triterpenes from *Pentadesma grandifolia* (Clusiaceae): Structural Determination and Bioactivity. *Natural Product Communications*, **5**, 1934578X1000500714 (2010) https://doi.org/10.1177/1934578X1000500714.

[33] Rufa'i, F. A., Baecker, D., & Mukhtar, M. D. Phytochemical Screening, GC-MS Analysis, and Evaluating In Vivo Antitrypanosomal Effects of a Methanolic Extract of *Garcinia kola* Nuts on Rats. *Antibiotics*, **12**, 713 (2023) https://doi.org/10.3390/antibiotics12040713

[34] Xu H-X, Mughal S, Taiwo O, Lee SF. Isolation and characterization of an antibacterial biflavonoid from an African chewing stick *Garcinia kola* Heckel (Clusiaceae). *Journal of Ethnopharmacology*, **147**, 497–502 (2013) https://doi.org/10.1016/j.jep.2013.03.047.

[35] Patterson S, Waters ME, Braman N, Willson R, Hill RA, Magolan J, Hofmann T, Stark TD, Balemba OB. *Garcinia buchananii* stem bark extract and its bioactive constituents Manniflavanone, GB-2 and Buchananiflavonone attenuate intestinal inhibitory neuromuscular transmission. *J Smooth Muscle Res*, **59**, 34-57 (2023) https://doi.org/10.1540/jsmr.59.34.

[36] Olatoye FJ, Akindele AJ. Ninety-day oral toxicological profiling of Kolaviron (an extract of *Garcinia kola*) in male and female rats. *Drug and Chemical Toxicology*, **46**, 1–14 (2023) https://doi.org/10.1080/01480545.2021.1997543.

[37] Abdullah I, Phongpaichit S, Voravuthikunchai SP, Mahabusarakam W. Prenylated biflavonoids from the green branches of *Garcinia dulcis*. *Phytochemistry Letters*, **23**, 176–9 (2018) https://doi.org/10.1016/j.phytol.2017.12.004.

[38] Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules*, **27**, 1149 (2022) https://doi.org/10.3390/molecules27041149.

[39] Chen J, He N, Wang Q, Wu G, Wu W, Xin Q, Cheng G, Sang Z, Zhu C, Wu Y, Wei R, Ma Q. A Comprehensive Review of Structures, Structure-activity Relationships, Extractions, and Bioactivities of Flavonoids from *Citrus medica*. *CCHTS*, **26**, 2411-23 (2023) https://doi.org/10.2174/1386207326666230330083136.

[40] Kumar A. Amentoflavone, a biflavonoid with potential anticancer properties against viral oncoprotein E6 in Human papilloma virus-16 (HPV-16) positive cervical cancer cells. *Natural Product Research*, 1–10 (2024) https://doi.org/10.1080/14786419.2024.2438273.

[41] Li X, Wang L, Han W, Mai W, Han L, Chen D. Amentoflavone Protects against Hydroxyl Radical-induced DNA Damage via Antioxidant Mechanism. *Turk J Bioch*, **39**, 30–6 (2014) https://doi.org/10.5505/tjb.2014.65882.

[42] Zhang G, Jing Y, Zhang H, Ma E, Guan J, Xue F, Liu H, Sun X. Isolation and Cytotoxic Activity of Selaginellin Derivatives and Biflavonoids from *Selaginella tamariscina*. *Planta Med*, **78**, 390–2 (2012) https://doi.org/10.1055/s-0031-1298175.

[43] Ayepola OR, Brooks NL, Oguntibeju OO. Kolaviron Improved Resistance to Oxidative Stress and Inflammation in the Blood (Erythrocyte, Serum, and Plasma) of Streptozotocin-Induced Diabetic Rats. *The Scientific World Journal*, **2014**, 1–8 (2014) https://doi.org/10.1155/2014/921080.

[44] Li X, Jiang Q, Wang T, Liu J, Chen D. Comparison of the Antioxidant Effects of Quercitrin and Isoquercitrin: Understanding the Role of the 6″-OH Group. *Molecules*, **21**, 1246 (2016) https://doi.org/10.3390/molecules21091246.

[45] Anaya-Eugenio GD, Blanco Carcache PJ, Ninh TN, Ren Y, Soejarto DD, Kinghorn AD. A pentamethoxylated flavone from *Glycosmis ovoidea* promotes apoptosis through the intrinsic pathway and inhibits migration of MCF -7 breast cancer cells. *Phytotherapy Research*, **35**, 1634–45 (2021) https://doi.org/10.1002/ptr.6930.

[46] Wätjen W, Weber N, Lou Y -j., Wang Z -q., Chovolou Y, Kampkötter A, Kahl R, Proksch P. Prenylation enhances cytotoxicity of apigenin and liquiritigenin in rat H4IIE hepatoma and C6 glioma cells. *Food and Chemical Toxicology*, **45**, 119–24 (2007) https://doi.org/10.1016/j.fct.2006.08.008.

[47] Chen B, Wang X, Zhang Y, Huang K, Liu H, Xu D, Li S, Liu Q, Huang J, Yao H, Lin X. Improved solubility, dissolution rate, and oral bioavailability of main biflavonoids from *Selaginella doederleinii* extract by amorphous solid dispersion. *Drug Delivery*, **27**, 309-22 (2020) https://doi.org/10.1080/10717544.2020.1716876.

[48] Liao S, Ren Q, Yang C, Zhang T, Li J, Wang X, Qu X, Zhang X, Zhou Z, Zhang Z, Wang S. Liquid Chromatography–Tandem Mass Spectrometry Determination and Pharmacokinetic Analysis of Amentoflavone and Its Conjugated Metabolites in Rats. J. *Agric. Food Chem*., **63**, 1957–66 (2015) https://doi.org/10.1021/jf5019615.

[49] Feng X, Chen Y, Li L, Zhang Y, Zhang L, Zhang Z. Preparation, evaluation and metabolites study in rats of novel Amentoflavone-loaded TPGS/Soluplus mixed Nanomicelles. *Drug Delivery*, **27**, 137–50 (2020) https://doi.org/10.1080/10717544.2019.1709920.

[50] Ghosh A, Banerjee T, Surolia A, Bhandary S. Formulation of nanotized curcumin and demonstration of its antimalarial efficacy. *IJN*, **5373** (2014) https://doi.org/10.2147/IJN.S62756.

[51] Zhao F, Qian Y, Li H, Yang Y, Wang J, Yu W, Li M, Cheng W, Shan L. Amentoflavone-loaded nanoparticles enhanced chemotherapy efficacy by inhibition of AKR1B10. *Nanotechnology*, **33**, 385101 (2022) https://doi.org/10.1088/1361-6528/ac7810.

[52] Yuan D, Guo Y, Pu F, Yang C, Xiao X, Du H, He J, Lu S. Opportunities and challenges in enhancing the bioavailability and bioactivity of dietary flavonoids: A novel delivery system perspective. *Food Chemistry*, **430**, 137115 (2024) https://doi.org/10.1016/j.foodchem.2023.137115.