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Original Research Article

Biflavonoid Anti-inflammatory Activity of the Araucariaceae Family—A Review

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ABSRTACT

The Araucariaceae family, a group of coniferous plants, has gained attention for its diverse bioactive compounds, particularly biflavonoids. These natural polyphenolic compounds have demonstrated significant anti-inflammatory properties, making them a promising target for pharmacological research. This review consolidates and critically analyzes the current knowledge on the anti-inflammatory activity of biflavonoids isolated from the Araucariaceae family. It explores their chemical structures, mechanisms of action, and potential therapeutic applications. Special attention is given to their ability to modulate key inflammatory pathways, including cytokine suppression, NF-κB inhibition, and antioxidant activity. Additionally, the review highlights challenges in harnessing these compounds for drug development, such as bioavailability and scalability, and discusses future directions in modern drug discovery. By providing a comprehensive overview, this study aims to bridge gaps in the literature and underscore the potential of Araucariaceae-derived biflavonoids in addressing inflammation-related diseases.

Keywords: Antioxidants, Araucariaceae, Biflavonoids, Inflammation therapy, Cytokine modulation, NF-κB inhibition.

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Introduction

Inflammation is the body's immune response to injury/infection. While a proper inflammatory response is essential for eradicating pathogens, it can experience imbalances that impact uncontrolled inflammation and lead to various diseases. Inflammatory markers indicate normal biological processes against pathogens.[1] Although the inflammatory response varies based on the type and location of the initial stimulus in the body, they all follow a standard process: 1) Recognition of harmful substances by cell surface receptors; 2) Activation of inflammatory pathways; 3) Release of markers indicating inflammation; and 4) Recruitment of inflammatory cells. Stimuli activate cells like macrophages and adipocytes, prompting the production of cytokines such as IL-1*β*, IL-6, TNF-α, and other inflammatory proteins and enzymes, which can potentially serve as biomarkers for inflammation.[2]

High levels of pro-inflammatory cytokines accelerate chronic inflammation, resulting in increasing immune system activation. This ongoing activation can lead to tissue damage and the development of cancer.[3] Inflammatory cytokines originate primarily from immune cells such as monocytes, macrophages, and lymphocytes.

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They fall into several categories: interleukins (IL), colony-stimulating factors (CSF), interferons (IFN), tumour necrosis factors (TNF), tumour growth factors (TGF), and chemokines that cells produce are secreted to attract leukocytes to sites of infection or injury. Proteins and enzymes involved in inflammation include superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), glutathione reductase (GR), glutathione-S-transferase (GST), NADPH oxidase (NOX), inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX). Additional inflammatory markers encompass increased oxidative stress, leading to reactive oxygen species (ROS) and malondialdehyde (MDA) production, which in turn activate various transcription factors like nuclear factor-kappa B (NF-κB), STAT1/STAT3, AP-1, Nrf2, HIF, and p53. This cascade enhances the expression of genes encoding growth factors, inflammatory cytokines, and chemokines.[4] Inflammation triggers the activation and infiltration of macrophages, which are responsible for eliminating oxidized lipids and damaged cells.[5]

In recent years, there has been an increase in literature discussing the classical anti-inflammatory signalling pathways associated with natural products. These products are advantageous because they target multiple pathways, offering a solid theoretical foundation for their potential as promising anti-inflammatory drugs. The slogan "back to nature" has gained significant traction, and for good reason. Herbal treatments, known for their safety and affordability, are becoming increasingly popular. Many chemical compounds found in plants, such as flavonoid.[6], are recognized for their ability to reduce inflammation. One notable flavonoid, biflavonoid, has been scientifically proven to exhibit anti-inflammatory activity, making it a reliable and effective treatment option.

Biflavonoids target several key inflammatory pathways, which help in reducing inflammation. Here are some of the primary pathways: (1) NF-κB Pathway: biflavonoids can inhibit the activation of the NF-κB pathway, which plays a crucial role in regulating the immune response to infection. By inhibiting this pathway, biflavonoids reduce the production of pro-inflammatory cytokines,[7] (2) COX-2 Enzyme: an enzyme responsible for forming pro-inflammatory prostaglandins. Biflavonoids can inhibit COX-2 activity, thereby reducing inflammation and pain, (3) nucleotide oligomerization domain-like

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receptor protein 3 (NLRP3) inflammasome: This is a multiprotein complex that plays a key role in the activation of inflammatory responses.[8] Biflavonoids have been shown to reduce the release of pro-inflammatory cytokines like IL-1β and IL-183,[9] and (4) mitogen-activated protein kinases (MAPK) pathway: MAPK are involved in transmitting signals from the cell surface to the DNA in the cell nucleus. Biflavonoids can inhibit the MAPK pathway, which reduces the production of inflammatory mediators.[10,11]

Biflavonoids are polyphenolic molecules arranged from two identical or different flavonoid units, linked together in symmetrical or asymmetrical arrangements through alkyl-based connectors or alcohol chains of varying lengths.[12] Biflavonoids, a subgroup within the flavonoid family, are only present in several plant species. They consist of dimeric structures where monomers are interconnected through C-C bonds (Figure 1a) or C-O-C bonds (Figure 1b), involving the flavonoid units A, B, or C rings. When the two structural subunits of flavonoids are the same, it characterizes the class of bisflavonoids, and when the subunits are different, it is a biflavonoid.[13] Chemical structures of most common biflavonoids are amentoflavone (1), agathisflavone (2), cupressuflavone (3), robustaflavone (4), ochnaflavone, and its derivatives.

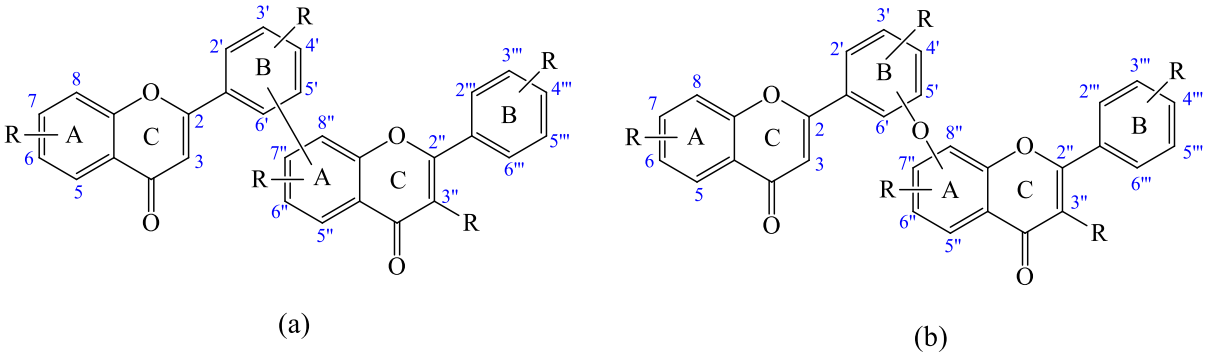

Figure 1: Chemical structure of biflavonoids featuring C-C linkages (a) and C-O-C linkages (b) [13]

Biflavonoids are natural compounds studied for their health benefits, particularly their ability to reduce inflammation. For instance, morelloflavone, found in *Garcinia spicata*, can inhibit an enzyme known as secretory phospholipase A2 (PLA2) in human studies, with an effective dose (IC_{50}) as low as 0.9 μM.[14] Additionally, ginkgetin (5) has been shown to decrease the levels of harmful substances called pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-8, in HeLa cells.[15] Ochnaflavone, derived from Lonicerae japonicae caulis, can lower the mRNA levels of TNF-α and IL-6 in macrophages stimulated with LPS,[16] although its absorption in the body needs improvement.[17] Lastly, 7,7"-di-O-methylamentoflavone, found in the leaves of Decussocarpus rospigliosii, specifically inhibits an enzyme called phosphodiesterase 4 (PDE4), showing promise for anti-inflammatory effects with an effective dose (IC_{50}) of 1.48 ± 0.21 μM.[18]

Biflavonoids derived from *Semecarpus anacardium* have demonstrated anti-inflammatory properties and potential antioxidant effects.[19] Notably, hinokiflavone (7) has been recognized as a possible anticancer agent.[20] Compound 1 is predicted to be an effective natural therapeutic drug candidate against SARS-CoV-2. Its strong antioxidant properties—along with anti-inflammatory effects, thrombin inhibition, and protection against lung injury—could help combat the pathogenic complications of SARS-CoV-2, such as hyperinflammatory and hypercoagulable states that can lead to acute lung injury (ALI) and multiorgan failure.[21] Additionally, compound 1 has shown promise in treating *Streptococcus suis* infections due to its antivirulence and anti-inflammatory qualities.[22] Bilobetin (8), sciadopitysin (9), and 7,4',7",4"'-tetra-*O*-methylamentoflavone (10) from *Cephalotaxus koreana* indicate therapeutic potential for bone diseases such as osteoporosis.[23] Moreover, 7,4',7"-tri-*O*-methylamentoflavone (11) from *Selaginella bryopteris* exhibited antiprotozoal activity with an IC_{50} of0.26 mM.[24] Finally, compound 2 from *Caesalpinia pyramidalis* Tull demonstrated antioxidant capacity[25]

The family that has been widely reported to contain biflavonoids is *Araucariaceae*. This family has three genera: *Agathis*, *Araucaria*, and *Wollemia*. These evergreen conifers are primarily distributed across the southern hemisphere's significant landmasses, excluding Southern Africa. Together, these genera encompass around 40 species. *Araucariaceae* is predominantly tropical, thriving in warm temperate regions of the Southern Hemisphere, especially in lowland rainforests of Malesia and New Caledonia. Additionally, species from this family can be found in Fiji, New Zealand, Australia, and South America.[26]

Biflavonoid compounds isolated from the Araucariaceae family have been widely reported; however, their potential as anti-inflammatory agents is still not well explored. Thus, it is important to investigate the anti-inflammatory effects of these biflavonoids, especially since multiple studies have indicated their potential therapeutic applications due to their anti-inflammatory activity. This review focuses on the Araucariaceae family, covering its general botanical features, the isolated biflavonoid compounds, and the pharmacological activities of biflavonoids both from Araucariaceae and other plant families that exhibit anti-inflammatory properties. Various retrieval systems were utilized to compile the data presented in this review.

Materials and Methods

This review was conducted by systematically collecting and analyzing relevant literature to assess the anti-inflammatory activity of biflavonoids from the Araucariaceae family. This review incorporated 103 articles spanning from 1969 to 2024, selected through an extensive literature search. The methodology involved the following steps:

Literature Search

Comprehensive searches were performed in scientific databases such as PubMed, Scopus, Web of Science, Publish and Perish, and Google Scholar using keywords including "Araucariaceae", "biflavonoids", and "anti-inflammatory activity" in the title, abstract and keywords.

Articles published up to [October 2024] were included, focusing on studies involving the isolation and characterization of biflavonoids from the Araucariaceae family and biological evaluation of biflavonoids, particularly their anti-inflammatory properties.

Inclusion and Exclusion Criteria

Studies were included if they reported on the chemical structure, mechanism of action, or biological activity of biflavonoids from the Araucariaceae family.

Articles were excluded if they lacked experimental evidence, were unrelated to biflavonoids or anti-inflammatory activity, or were in non-peer-reviewed sources.

Data Extraction

Key information, including plant plant sources, biflavonoid structures, experimental methods, and anti-inflammatory effects, was extracted and organized into a Table. Mechanisms of action such as cytokine modulation, inhibition of NF-κB signalling, and antioxidant activity were highlighted.

Data Synthesis

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A comparative analysis was conducted to identify biflavonoids isolated from the Araucariaceae family, based on 30 articles published between 1969 and 2024. The study of these biflavonoids was performed using Orange software, along with the free Viz tool (S1), to evaluate the abundance and distribution of various biflavonoids within the Araucariaceae family. Additionally, their inflammatory mechanisms were examined in vitro, in vivo, and in silico models..

Critical Evaluation
Challenges related to bioavailability, scalability, and clinical application were discussed. Future research directions were proposed based on identified gaps and advancements in the field. This systematic approach ensures a comprehensive and balanced review of the anti-inflammatory potential of biflavonoids from the Araucariaceae family.

Results and Discussion

Biflavonoids from the Araucariaceae family

The biflavonoids identified in *Araucariaceae* plants have 46 compounds, including compounds 1, 2, 3, and 4 with their derivatives, 7, 2',8''-biapigenin (12), 2'',3''-dihydro-3',3'''-biapigenin (13), and isocryptomerin (14). The 46 compounds were processed to see the distribution of the abundance of biflavonoid compounds identified in each genus, presented in (Figure 2), It is implemented by Orange software. Orange is an open-source machine learning and data mining software, written in Python for interactive data analysis and component-based construction of data mining methods.[27] The compounds of 7-*O*-methylagathisflavone (15) and 7,7''-di-*O*-methylcupressuflavone (16) are most commonly found in several species in the genus *Agathis*, whereas 7,4′,7″,4‴-tetra-*O*-methylcupressuflavone (17), is most abundant in *Araucaria* plant, and 7,4'''-di-*O*-methylagathisflavone (18) was abundant in *Wollemia nobilis*. On the other hand, compounds 2, 3, 15, 16, 17, 18, 7-*O*-methylcupressuflavone (19), and 7,4',7''-tri-*O*-methylcupressuflavone (20), were found in all genera of the Araucariaceae. For a complete list of all the biflavonoid compounds identified in the genera *Agathis, Araucaria, and Wollemia*, please refer to Table 1. Their respective structures are illustrated in Figure 3.

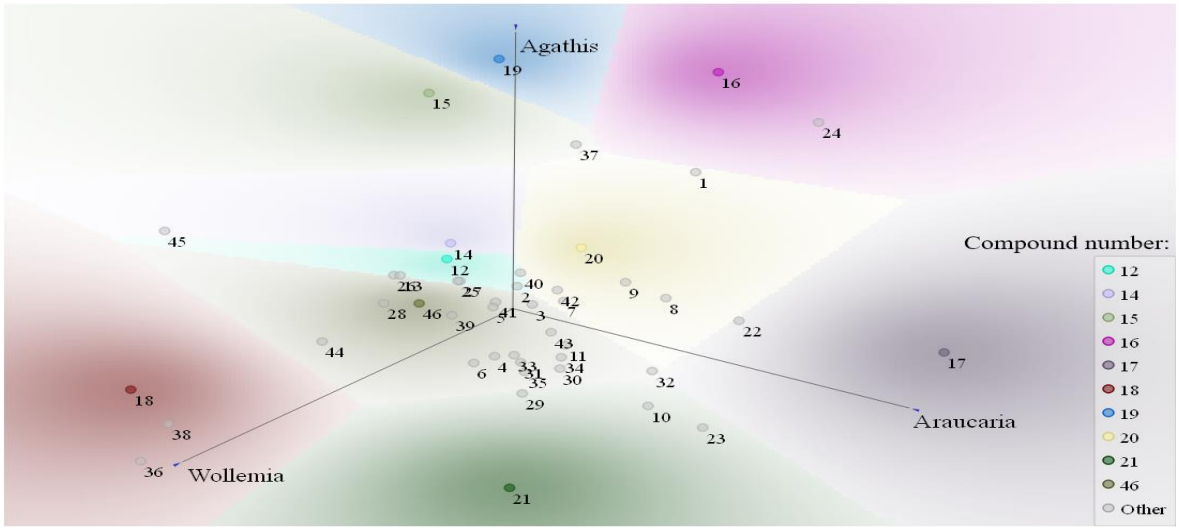

Figure 2: The distribution of biflavonoids in the *Araucariaceae* family.

Table 1: Biflavonoid compounds isolated from *Agathis, Araucaria* and *Wollemia* Genus

Compound No.	Genus *Agathis*									Genus *Araucaria*								Genus *Wollemia*					
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w
1		□						□	□	□		□					□						
2		□				□						□					□				□		
3		□				□				□		□					□				□		
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5								□		□													
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7								□				□											
8								□		□		□											
9								□		□							□						
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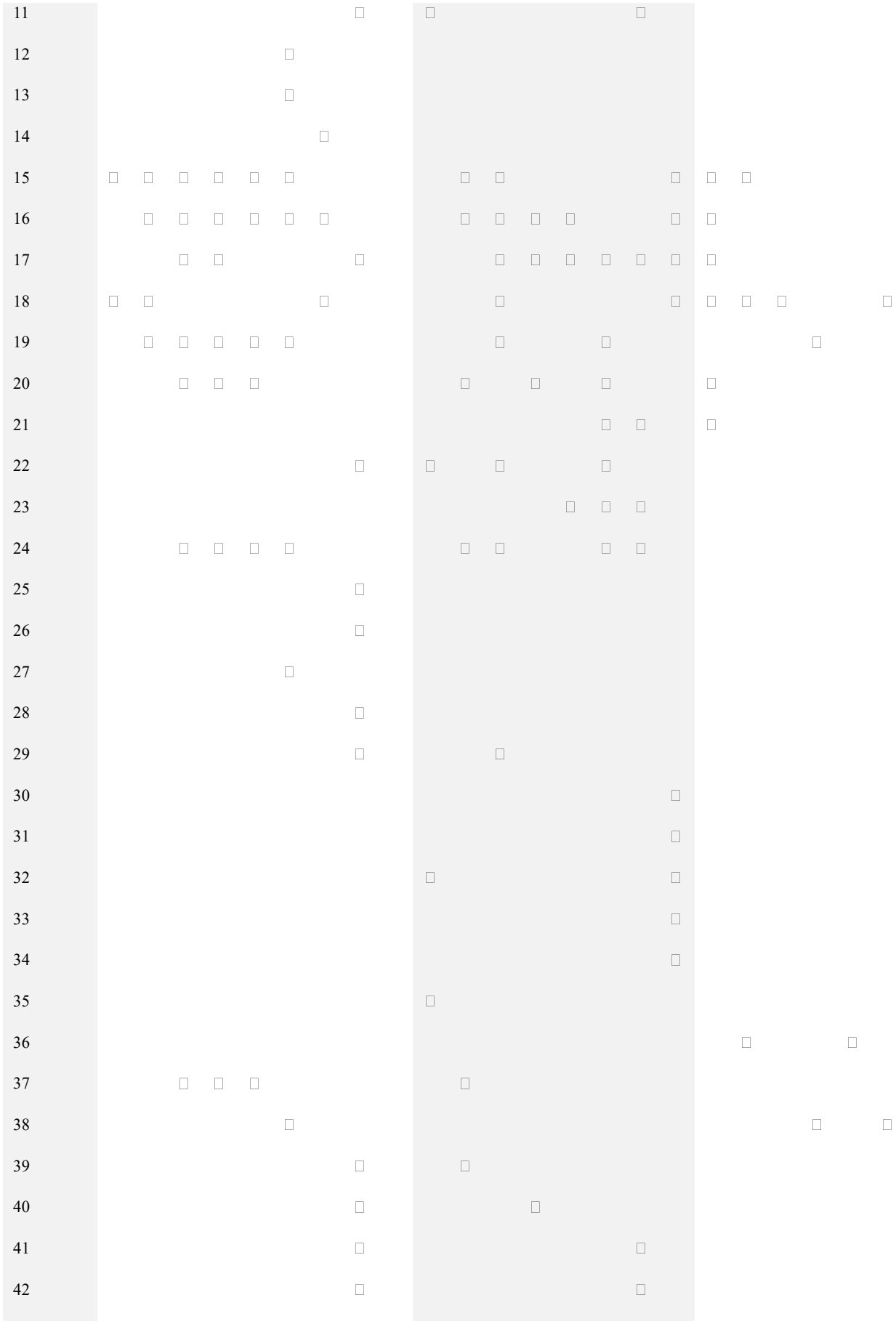


43					
44					
45					
46					

Note:

a	*Agathis pulmerstoniil*[28]	j	*Araucaria angustifolia* [37-39]
b	*Agathis alba*[29,30]	k	*Araucaria araucana*[40]
c	*Agathis australis* [31]	l	*Araucaria bidwillii*[30,41,42]
d	*Agathis ovata*[31]	m	*Araucaria cunninghamii*[43-45]
e	*Agathis atropurpurea*[31]	n	*Araucaria columnaris*[45,46]
f	*Agathis robusta*[31,32,33]	o	*Araucaria hunsteinii*[12,49]
g	*Agathis microstachya*[34]	p	*Araucaria heterophylla*[50,51]
h	*Agathis dammara*[35]	q	*Araucaria rulei*[52]
i	*Agathis macrophylla*[36]	r-w	*Wollemia nobilis*[26,53-57]

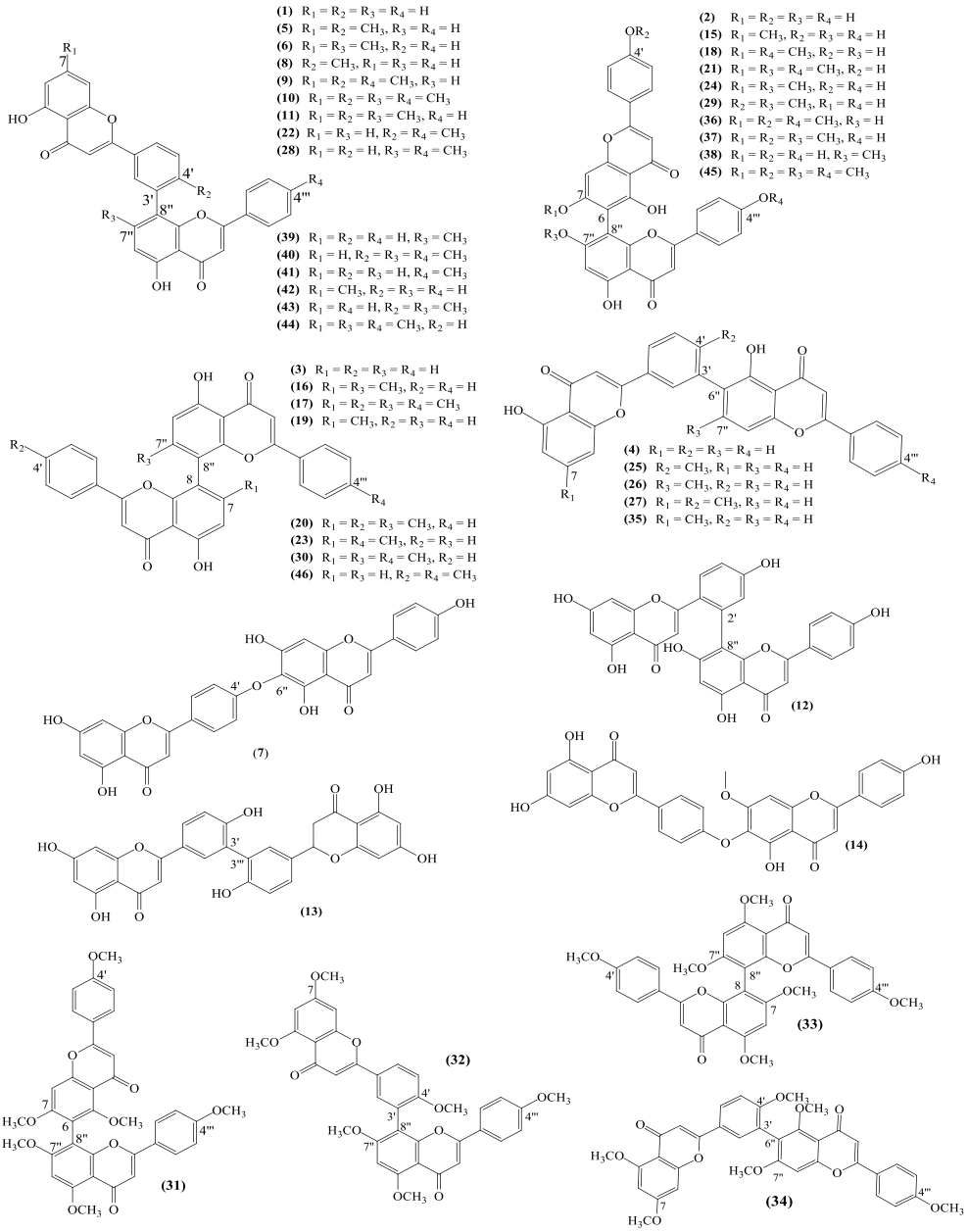


Figure 3: Biflavonoids structure from the *Araucariaceae* family

The family Araucariaceae has been studied for its biflavonoid content and bioactivity. Compounds such as compound 20 and 7,7",4"'-tri-*O*-methylagathisflavone (21), derived from *Araucaria hunsteinii*, exhibit promising anticancer activity against MCF-7 cells.[27] Additionally, compounds 19 and 4',4"'-di-*O*-methylamentoflavone, also known as 4',4"'-di-*O*-methylamentoflavone/isoginkgetin (22), act as inhibitors against both HeLa and MCF-7 cancer cells.[58] Further investigations have revealed that compounds 19, 21, 22, 7,4"'-di-*O*-methylcupressuflavone (23), and 7,7"-di-*O*-methylagathisflavone (24) demonstrate antidiabetic activity in vitro and through in silico model.[59] Compounds 20 and 23, isolated from the leaves of *Araucaria columnaris*, exhibit antiangiogenic activity against calf pulmonary arterial endothelial (CPAE) cell.[46] Moreover, the biflavonoid fraction (BFF) extracted from the needles of *Araucaria angustifolia* showed the ability to protect calf thymus DNA from damage caused by UV radiation.[60] Antimicrobial activity has been observed in compounds 17 and 20 derived from *Araucaria cunninghamii*.[43]

Genus Agathis

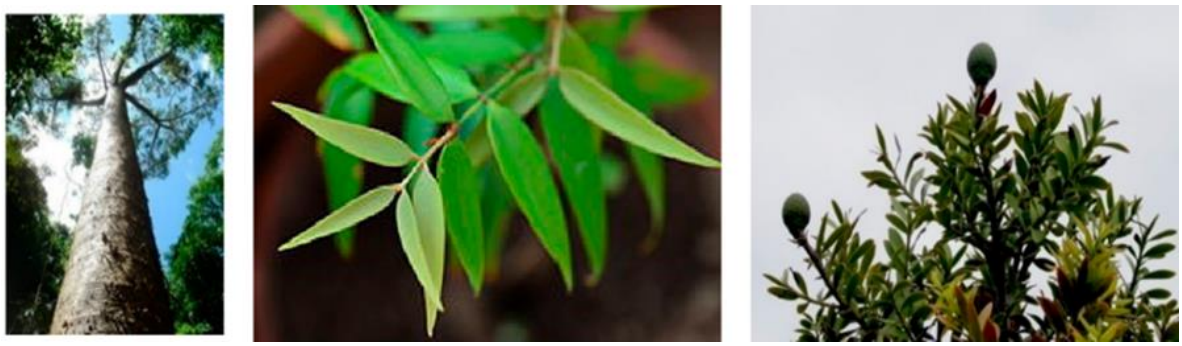
Agathis, a genus of broadleaved conifers in the *Araucariaceae* family, includes approximately 17 living species. These trees have historically been prevalent in various regions, ranging from lowland to upper montane rainforests, spanning from Sumatra to New Zealand. *Agathis* is a widely distributed genus within its family. Its species are found in regions such as New Zealand, the Philippines, New Guinea, Melanesia, and Australia, extending into Malaysia beyond the equator. They thrive on diverse substrates, including podzolized sands, ultramafics, carbonates, and silicates. Their habitat ranges from near sea level up to approximately 2500 meters altitude.

These trees typically prefer frost-free environments receiving five to ten meters of annual rainfall. They are recognized by their large, sturdy trunks that remain unbranched at the lower part when mature, though they start as conical shapes with irregular crowns when young. The bark is smooth, grey-brownish coloured, peeling in irregular flakes that thicken with age. Branches often grow horizontally or ascend when mature, leaving circular scars upon detachment from the trunk. Juvenile leaves are larger and more acute, shaped ovate to lanceolate. *Agathis* produces two cones: pollen-bearing male cones found only on most giant trees and seed-bearing female cones typically developing on short lateral branches, maturing over two years, usually oval or globe-shaped (Figure 4).[61] *Agathis* species have been reported for biological activity, such as leaf essential oil from *A. dammara* has a potential for anti-melanogenesis,[62] and antibacterial activity[63]. *A. robusta* has been investigated for antibacterial activity,[64] and hepatoprotective activity.[65]

Several studies have reported biflavonoid content in the genus *Agathis* (Table 1). However, what sets our research apart is the discovery of the compound that is only found in the *Agathis* genus in the *Araucariaceae* family, such as compounds 12, 13, 14 4'-*O*-methylrobustaflavone (25), 7″-*O*-methylrobustaflavone (26), 7,4'-di-*O*-methylrobustaflavone (27), and 7″,4"'-di-*O*-methylamentoflavone (28) found in *A. dammara*.[35] On the other hand, in *A. robusta* leaves that grow in Australia and Italy there are differences in content, for example, the compound 16 was identified in *A. robusta* leaves grown in Australia but not identified in *A. robusta* leaves grown in Italy. [31,33]

Genus *Araucaria*

Araucaria is a genus of 19 species of pine-like coniferous trees that belong to the Araucariaceae family.[66] These trees can be found in various regions, including Brazil, Chile, Argentina, Papua, New Caledonia, Norfolk Island, Indonesia, and Australia. The name "Araucaria" comes from Arauco, a town in southern Chile where these trees were first discovered. New Caledonia is home to the greatest diversity of Araucaria species, which thrive in ultra-alkaline and calcareous schistose (massive) soils. Most species in this genus are dioecious, meaning they have separate male and female individuals. However, some species are monoecious, having both male and female reproductive structures on the same tree. Interestingly, some Araucaria trees can change sex over time. These remarkable trees are characterized by their large, upright trunks that can reach heights of up to 80 meters. (Figure 5).[67]



(a) (b) (c)

Figure 4: Images of *Agathis* species*: A. microstachya* trunk (a); *A. philippinensis* leaves (b); *A. australis* leaves and cones (c)[61]

(a) (b) (c)

Figure 5: Images of *Araucaria columnaris*: aerial parts (a), bark (b) and leaves (c)[67]

The branches are gathered in a circle and grow horizontally covered with coarse leaves or needles without branching in the inferior part when they are mature. In some species, the leaves are narrow and lanceolate, almost not overlapping, while in others, the leaves are extensive, flat, and heavily overlapping. The plant produces two cones (*cones*), male (pollen) and female (seeds).[61] The genus *Araucaria* contains a variety of phytochemical compounds. Biflavonoids, diterpenoids, phenylpropanoids, and lignans are abundant in this genus. *A. angustifolia* and *A. heterophylla* are rich in biflavonoids, while *A. araucana* is rich in terpenoids.[68] The pharmacological of *Araucaria* species have been reported such as antivirus,[48] antipyretic, antiviral, antimutagenic, antinociceptive antioxidant,[69] and antifungal[70] activity from *A. angustifolia* extract. Hamed et al. (2019)[71] reported that *A. heterophylla* has ~~a~~ potent antioxidant, anticancer and antimicrobial activities. *A. araucana* crude extract may exhibit antispasmodic activity, bronchodilation, and vasodilation by inhibiting voltage-dependent Ca^{++} channels and release of subcellular calcium.[72]

The biflavonoid content of eight of 19 species of the genus *Araucaria*, each with its own unique set of compounds, has been reported (Table 1). Among these, compounds that are exclusively found in the genus *Araucaria* of the *Araucariaceae* family are 4',7"-di-*O*-methylagathisflavone (29) in the leaves of *A. bidwilli*,[41] compound 6 in the leaves of *A. heterophylla*,[51] compound 24 in the leaves of *A. Araucana*, [40] *A. bidwillii*,[30] *A. hunsteinii*,[12,49] and *A. heterophylla*,[51] as well as the compound 4, 7,7",4'"-tri-*O*-methylcupressuflavone (30), hexa-*O*-methylagathisflavone (31), hexa-*O*-methylamentoflavone (32), hexa-*O*-methylcupressuflavone (33) and hexa-*O*-methylcupressuflavone (34) found in the leaves of *A. rulei*,[52] and 7-*O*-methylrobustaflavone (35) in the leaves of *A. angustifolia*.[38,39]

Based on geographical sources, *A. bidwillii* leaves also have different biflavonoid compound contents based on where they grow in India, Egypt, and the Philippines. *A. bidwillii* leaves from India contain, compound 16, while those from Egypt and the Philippines do not. In addition, only *A. bidwilli* leaves from the Philippines were identified as compound 17.[30,41,42] The leaves of *Araucaria columnaris* that grow in Indonesia and Italy have similarities in containing the compound 17.[45,46] Besides that, compound 17 was also found in *A. cunninghamii* leaves from India and Italy.[43-45]

Genus Wollemia

Wollemia is a genus comprising a single, scarce species. All information regarding this genus, including its physical description, geographical range, chemical composition, traditional uses, and medical properties, relates exclusively to its lone species, *Wollemia nobilis* W.G. Jones, K.D. Hill and J.M. Allen. Indigenous to Australia, this species is highly uncommon, growing naturally in just three locations within New South Wales' Wollemi National Park. There are approximately 20 mature trees, reaching up to 40 meters, alongside 20 juvenile specimens. Thought to be extinct until its rediscovery in 1994, extensive efforts have since been undertaken to protect and conserve it. These measures include maintaining secrecy about its habitat, monitoring against unauthorized visits, and promoting global cultivation. As a result, numerous *W. nobilis* specimens are now housed in botanical gardens worldwide, including Italy, as well as in thousands of private Australian gardens.

The species thrives in well-drained sandy soils with regular watering. These monoecious trees can reach up to 40 meters in height and often regenerate from their bases. Their slender columnar crowns are at their widest, about a third of their overall height. The bark peels into fragile dark red-brown scales on young branches. However, on older trunks, it transforms into soft, spongy nodules up to 10 millimetres in diameter and 15 millimetres in length, forming a layer as deep as 20 millimetres. *W. nobilis* is classified within the *Araucariaceae* family, distinguished by its broad leaves with numerous parallel veins and the absence of a precise central vein, microsporophylls that each holds 4-9 pendulous microsporangia, pollen lacking wings, and prominent female cones containing numerous fully fused bract-scale complexes, each bearing a single inverted ovule maturing into a dry, winged seed. Unlike *Araucaria* species, it features trimorphic leaves that are generally obtuse or rounded, fully fused bract and ovuliferous scales that lack an apparent vestigial scale tip, and seeds shed independently of their scales(Figure 6).[73] The ethnopharmacological uses and biological activities of *W. nobilis* extract have not been reported. The preliminary ethnopharmacological and nutraceutical evaluation of *W. nobilis* cones showed a good potentiality, also supported by the semi-quantitative analysis.[57]

(a) (b) (c)

Figure 6: Images of Wollemia nobilis: tree (a)[61], leaves (b)[56] cone (c)[61].

Table 1 shows the biflavonoid content found in various parts of *W. nobilis*. Compound 18 was detected in twigs from Poland,[26] and in the male reproductive organs, [53] male cones,[54] and half-mature female cone[57] parts collected from Italy. Additionally, the compound 7,4′,4‴-tri-*O*-methylagathisflavone (36) was found in both the male reproductive organs[53] and the leaves.[56] This compound is unique to *W. nobilis*, which belongs to the Araucariaceae family. Compound 7,4',7"-tri-*O*-methylagathisflavone (37), 7"-*O*-methylamentoflavone/sotetsuflavone (39), 4′,7″,4‴-tri-*O*-methylamentoflavone/ kayaflavone (40), 4'"-*O*-methylamentoflavone/podocarpusflavone A (41), 7-*O*-methylamentoflavone/sequoiaflavone (42), 4′,7″-di-*O*-methylamentoflavone (43), and 4′,4‴-di-*O*-methylcupressuflavone (46) was not identified in the genus *Wollemia.* While compounds 7″-*O*-methylagathisflavone (38), 7,7″,4‴-tri-*O*-methylamentoflavone/heveaflavone (44), and 7,4′,7″,4‴-tetra-*O*-methylagathisflavone (45) was only identified in the genera *Agathis and Wollemia*. ,

Anti-inflammatory Activity

The anti-inflammatory activity of the extract from *Agathis robusta* was studied. The aqueous extract of its leaves was tested at 200 µg/ml and 400 µg/ml concentrations. It was compared with standard doses of diclofenac (20 µg/ml and 40 µg/ml) in the HRBC membrane stabilization model. The same extract concentrations were tested against aspirin (200 µg/ml) using the heat-induced hemolytic method. Promising results were observed with the extract at a 400 µg/ml dose in both in vitro modesl.[74] In the in vivo renal ischemia-reperfusion injury

experiments, pretreatment of the etanolic extract of *Agathis robusta* bark improved kidney function and structural changes. The extract lowered renal expression of p-NFκB and cleaved caspase-3 in rats subjected to renal ischemia-reperfusion injury by suppressing HSP90 and P53.[33]

A study investigated the anti-inflammatory potential of methanolic extracts from three species of Araucaria found in Egypt: *A. cunninghamii*, *A. bidwillii*, and *A. heterophylla*. The researchers evaluated the anti-inflammatory activity of these extracts by measuring the percentage inhibition of prostaglandin E2 (PGE2) production. The results indicated that *A. cunninghamii* and *A. bidwillii* exhibited the highest levels of activity, with IC_{50} values of 23.20 ± 1.17 µg/mL and 82.83 ± 3.21 µg/mL, respectively. In contrast, *A. heterophylla* had a higher IC_{50} value of 221.13 ± 6.7 µg/mL. For comparison, the standard drug Celecoxib had an IC_{50} value of 141.92 ± 4.52 µg/mL.[75]

According to Talaat et al. (2018),[41] both the entire methanol extract and the polyphenol-enriched fraction from *Araucaria bidwillii* leaves showed a dose-dependent effect in reducing TNF-α, IL-6, and IL-1β levels in PHA-stimulated PBMCs, similar to the effects of indomethacin. *Araucaria bidwillii* has been found in several studies to have potential anti-inflammatory properties. In a survey by Abdelhameed et al., the essential oil extracted from *A. bidwillii* shoots, and its nanoemulsion formulation effectively reduced inflammation in a rat model of carrageenan-induced paw edema. This effect was observed through oral administration (at doses of 50 and 100 mg/kg) and topical application (5% in soybean oil), compared to control and reference drug groups. The study showed significant decreases in inflammatory markers such as IL-1β and IL-8, as well as reductions in nitrosative stress (NO) PGE2, as confirmed by histopathological analyses and immunohistochemical evaluation of MMP-9 and NF-κB levels in paw tissues.[76]

Ahamed et al. investigated ethanol extracts of *A. bidwillii* leaves using the same approach. They found that the extract significantly inhibited carrageenan-induced (18.61%, 32.12%, and 45.64%) and serotonin-induced (32.81%, 38.68%, and 40.75%) hind paw oedema in rats at doses of 100, 200, and 300 mg/kg of the *A. bidwillii* extract, respectively.[41] The anti-inflammatory effects observed with the extract were comparable to those of the standard drug indomethacin at 5 mg/kg (68.51% and 63.28%). Mukherjee et al. in Patial and Cannoo[77] found that a biflavone-rich extract from *A. bidwillii* leaves protected the rat brain from oxidative stress caused by ischemia/reperfusion. The biflavone fraction, administered at 100 and 200 mg/kg doses, showed a protective effect similar to that of vitamin E (200 mg/kg). Additionally, pre-treatment with higher doses of biflavones notably reduced the ischemia-induced neuronal loss in the brain, aligning with improvements in neurobehavioral deficits.

On the other hand, the methanol extract of *A. bidwillii* and *A. excelsa* oleo-resin (100, 200 and 400 mg/kg) reduced carrageenan-induced paw oedema in rats.[78] The essential oil of *Araucaria heterophylla* resin (100 mg/kg) administration in the rat paw oedema and rectal temperature exhibited a significant difference ($P < 0.05$) by 32 %, compared to indomethacin (39 %). Also, it attenuated the levels of proinflammatory cytokines (TNF)-α, IL-6, and IL-1 β by 201.25, 285.62 and 437.0 pg/ml, respectively. Further, the administration of the higher dose of EO and its emulsion (200 mg/ kg) attenuated the levels of inflammatory cytokines, and improved paw oedema and rectal temperature in rats. At the same time, the results showed that the low dose of nanoemulsion of *Araucaria heterophylla* resin (100 mg/kg) was the least effective.[79]

Anti-inflammatory Activity of Biflavonoid

Research on the anti-inflammatory activity of biflavonoid compounds from the *Araucariaceae* family is still scarce. Biflavonoids from the plant *Selaginella uncinata, Juniperus rigida, Metasequoia glyptostroboides, Capparis spinosa, Ginkgo biloba, Poincianella pyramidalis, Cenostigma pyramidale, Taxus x media var. Hicksii, Cupressus macrocarpa, Nandina domestica*, *Camellia oleifera*, *Ouratea spectabilis*, *Garcinia kola*, and *Canarium Album* have reported anti-inflammatory activity using in vitro and in vivo models. Biflavonoids modulate a transcription factor that suppresses the expression of numerous genes induced by cytokines, including compound 1 and its derivatives. Compound 1 affects epileptogenic and exerts neuroprotective effects by inhibiting the NLRP3 inflammasome and, thus, mediating the inflammatory process in PTZ-induced kindling mice and LPS-induced BV2 microglial cells. Therefore, compound 1 may be a potential treatment option for epilepsy.[9] Compound 1 reversed the activation, migration, and inflammatory response of LPS-induced microglia by modulating the toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/NF-κB pathway. Additionally, compound 1 increased the levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) in BV2 microglial cells treated with LPS.[80] In lipopolysaccharide-induced RAW264.7 macrophages, bilobetin (8) and isoginkgetin (22) from *Ginkgo biloba* L. had significant dose-dependent inhibitory effects on TNF-α, IL-6, PGE2, inducible NO synthase mRNA, and cyclooxygenase-2 mRNA levels.[81]

Compound 1 from *Torreya nucifera* effectively reduces macrophage-mediated inflammatory responses, including the production of NO and PGE2. Specifically, it significantly blocks the nuclear translocation of c-Fos by inhibiting its upstream signalling enzyme, extracellular signal-regulated kinase (ERK).[82] Compound 1 isolated from the roots of *Cnestis ferruginea* produced an anti-neuroinflammatory lipopolysaccharide (LPS)-induced neuroinflammatory cascade of events associated with the oxidative and nitrative stress, and TNF-a production in rat astrocytoma cell line (C6) and human monocytic leukaemia cell line (THP-1).[83] Tetrahydroamentoflavone and compound 1 isolated from *Canarium Album* L. fruit as the compounds responsible for the anti-inflammatory activity exhibited an effect by inhibiting the production of NO using lipopolysaccharide (LPS)-stimulated mouse macrophages.[84]

The anti-inflammatory effects of biflavonoids isolated from caper (*Capparis spinosa*) fruits were evaluated using a secreted placental alkaline phosphatase (SEAP) reporter assay, which measures the activation of NF-κB. In the initial screening at a concentration of 20 µM, compounds 5 and 22 demonstrated inhibitory effects, with compound 5 showing significantly stronger inhibition than compound 22. A subsequent dose-response analysis determined the IC_{50} value for compound 5 to be approximately 7.5 µM, indicating its potential as a strong NF-κB inhibitor and warranting further investigation in vivo.[85] Additionally, the PI3K/Akt pathway is crucial for the expression of COX-2 and plays a significant role in inflammation. This pathway is also important for the expression of matrix metalloproteinase (MMP)-9. Compound 22, isolated from *Metasequoia glyptostroboides*, significantly reduced MMP-9 expression and inhibited invasive properties by targeting this pathway. Therefore, compound 22 shows promise as a potential therapeutic agent for addressing tumour invasion.[86]

In another study, Lim et al. reported compound 5 isolated from *Ginkgo biloba* leaves, was assessed both in vitro and in vivo. Topically applied to the ears of ICR mice at doses ranging from 20 to 80 µg per ear per treatment, ginkgetin inhibited ear oedema (22.8% to 30.5%) and reduced prostaglandin E2 production (30.2% to 31.1%) induced by repeated treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA) over 7 days.[87] Compound 5 also suppressed the expression of the proinflammatory gene IL-1β. Son et al. in Al-kuraishy et al.[88] reported compound 5 effectively suppresses the COX-2-dependent phases of prostaglandin D2 (PGD2) generation in bone marrow-derived mast cells (BMMC) in a manner that depends on its concentration, with IC_{50} values measured at 0.75 mM. Analysis using specific anti-COX-2 antibodies in Western blotting demonstrated a reduction in both PGD2 production and COX-2 protein levels. Furthermore, this compound consistently inhibited the production of leukotriene C4 (LTC4) in a dose-dependent manner, with an IC_{50} value of 0.33 mM. These results indicate that ginkgetin possesses dual inhibitory activity against COX-2 and 5-LOX.

Kwak et al. (2002) in Kim (2022)[87] investigated compound 5 exhibited a dose-dependent inhibition in an animal model of acetic acid-induced writhing. The ED_{50} values for compound 5 and indomethacin were 8.9 mg/kg and 3.8 mg/kg, respectively. These findings suggest that compound 5 has potential as an antiarthritic agent with analgesicactivity. Compound 5 at concentrations ranging from 1-10 µM, along with a biflavonoid mixture (10-50 µg/ml) primarily composed of equal parts compound 5 and 22 from *G. biloba* leaves,

inhibited the production of prostaglandin E2 in RAW 264.7 cells induced by lipopolysaccharide. Furthermore, compound 5 was shown to reduce COX-2 levels in the dorsal skin of ICR mice treated with TPA. Biflavonoid from *G. biloba* inhibited the enzyme of cAMP-Phosphodiesterase in rat adipose tissue with order compound 1 is strongly active followed by compound 8, 42 and 5, whereas compound 9 is inactive (Saponara and Bosisio in Thao et al).[89] These findings are significant as they provide a deeper understanding of the potential therapeutic properties of biflavonoid from *G. biloba* particularly in the context of current research on inflammation and pain management.

Compound 2 isolated from *Poincianella pyramidalis* (Tul.) leaves effectively reduced cell death caused by glutamate. This effect was associated with decreased levels of pro-inflammatory (M1) microglial cytokines TNFα, IL-1β, and IL-6, contributing to neurotoxicity. Furthermore, there was an increase in the expression of anti-inflammatory (M2) markers IL10 and arginase 1, which are associated with neuroprotective actions by microglia. Additionally, compound 2 was found to enhance the levels of neuroprotective trophic factors such as BDNF, NGF, NT4, and GDNF.[90] Compound 2 from *Poincianella pyramidalis* (Tul.) protects against the damaging effects caused by IL-1β, a critical cytokine released by activated microglia and astrocytes. Furthermore, qPCR analysis revealed that compound 2 reduced the expression of proinflammatory molecules TNF-α, IL-1β, connexin CCL5, and CCL2 while increasing the expression of IL-10 regulatory molecules. These results collectively suggest that compound 2 has significant neuroprotective and anti-inflammatory activity in vitro, demonstrating its potential as a supportive treatment for neurodegenerative diseases.[91]

Most microglia exposed to compound 2 from *Cenostigma pyramidale* (Tul.) showed an anti-inflammatory response characterized by increased CD206 expression and a branched morphology. This was associated with reductions in NO levels, GSH mRNA related to the NRLP3 inflammasome, as well as decreases in IL-18, IL-1β, IL-6, TNF, CCL2, and CCL5.[92] Compound 24, a biflavonoid isolated from the needles of *Taxus x media var. Hicksii*. Compound 24 inhibited TNF-α, IL-1β, and IL-6 production in LPS-induced macrophages. This compound also inhibited inflammatory macrophage migration by downregulating the gene and protein expression of adhesion molecules (LFA-1 and VLA4) and regulators of actin assembly (Cdc42-Rac1 pathway).[93]

Compounds 3, 4, and their derivatives have been shown to have anti-inflammatory and antioxidant properties. Compound 3 from *Camellia oleifera* shells had substantial free radical scavenging action in vivo and significantly reduced malonaldehyde (MDA) while increasing SOD and glutathione peroxidase (GSH-Px) activity in blood ($p < 0.01$). It suggests that by removing free radicals, biflavonoid can reduce prostaglandins and suppress mediators, so controlling inflammation and discomfort.[94] In the oxygen radical absorbance capacity assay, morrelloflavone from *Garcinia madruno* demonstrated robust reactive oxygen species (ROS) scavenging properties. More significantly, they shielded low-density lipoprotein particles from oxidation of both lipids and proteins.[95] In a model of inflammation induced by carrageenan in the paw oedema, compound 3 from the leaves of *Cupressus macrocarpa* demonstrated anti-inflammatory properties. It significantly reduced paw oedema by 55%, 60%, and 64% at doses of 40 mg/kg, 80 mg/kg, and 160 mg/kg when administered orally, respectively. Compound 3 also decreased levels of pro-inflammatory markers in plasma: PGE2 by 44%, 54%, and 58%; TNF-α by 26%, 37%, and 53%; IL-1β by 19%, 33%, and 41%; and IL-6 by 32%, 44%, and 55% across the tested doses. The highest dose exhibited effects similar to diclofenac sodium (100 mg/kg).[96] Adding compound 3 to the diet decreased pro-apoptotic markers and boosted anti-inflammatory and anti-apoptotic markers. Compound 3 is a potent anti-apoptotic compound effective against doxorubicin-induced hepatic toxicity.[97]

Compound 4 from *Nandina domestica* fruits inhibits the expression of iNOS and COX-2. It also decreases NF-κB expression induced by LPS and suppresses the phosphorylation of extracellular signal-regulated kinases (pERK 1/2). Additionally, compound 4 reduces the release of IL-8 in LPS-induced human colon epithelial cells (HT-29). These findings indicate that robustaflavone could be an effective treatment for inflammatory bowel disease (IBD).[98] Compound 25 exhibited comparable inhibition of LPS-induced lung oedema and neutrophil infiltration, along with elevated levels of IL-6, TNF-α, P-selectin, and ICAM-1 in the serum of LPS-challenged mice. Additionally, compound 25 isolated from *Selaginella uncinata* effectively suppressed inducible neutrophil activation in a concentration-dependent manner. It also reduced intracellular calcium levels and downregulated the expression of CCR2. Rescue experiments demonstrated that compound 25 inhibited FLT3 and its downstream targets p-p38 and p-AKT, an effect partially reversed by FLT3 agonist FLT3L, and to a lesser extent by mitogen-activated protein kinase (MAPK) agonist PDBu or AKT agonist SC79.[99]

Biapigenin (98% purity) suppressed the production of TNF-α, NO, IL-1β, and macrophage inflammatory protein (MIP)-2 cytokines. It inhibited the expression of IL-1β, iNOS, and MIP-2 mRNA, and partially reduced MIP-1 and TNF-α mRNA levels. Moreover, biapigenin significantly attenuated the increase in p38 MAPK phosphorylation induced by LPS. These results suggest that biapigenin is a potent biflavonoid inhibitor of the p38 MAPK pathway, highlighting its potential therapeutic utility in treating inflammatory diseases.[10] New (3,3″)-linked biflavanone-*O*-methyl ethers named ouratein D isolated from *Ouratea spectabilis* inhibited in vitro the release of the pro-inflammatory cytokine CCL2 by lipopolysaccharide-stimulated THP-1 cells (IC_{50} of 3.1 ± 1.1 μM), whereas TNF and IL-1β release was not reduced by any of the biflavanone.[100]

Kolaviron, a natural biflavonoid extracted from the seeds of *Garcinia kola*, has demonstrated anti-inflammatory properties. Treatment with kolaviron (100 mg/kg) significantly improved hyperglycemia and liver dysfunction. Serum levels of hepatic marker enzymes were notably reduced in diabetic rats treated with kolaviron. Additionally, kolaviron effectively prevented the diabetes-induced increase in hepatic levels of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and monocyte chemotactic protein (MCP-1).[101] In a separate study by Park et al. in He et al.,[102] the effects of six synthetic C-C biflavonoids, differing in the positions of the C-C bonds between flavone monomers (4′–4′, 4′–3′, 4′–6, 3′–6, 6–6, and 4′–3), were examined on the production of PGE2 and nitric oxide (NO) from lipopolysaccharide (LPS)-treated macrophages. Among these, the biflavonoid with the 6-6 bond exhibited the most potent inhibitory effect on PGE2 production, with an IC_{50} of 3.7 μM. In contrast, compound 5 (a natural biflavonoid) had an IC_{50} ranging from 8.2 to 20.7 μM.

Biflavonoid has been studied for its anti-inflammatory properties through in silico approaches. Molecular interactions and binding efficiency of compound 2 analyzed against 17 biomacromolecules revealed that the biflavonoid exhibited the best interaction with iNOS and COX-2 enzymes efficiently for its anti-inflammatory effects.[103] Molecular modelling studies indicated that compound 29 exhibited the strongest binding affinity for TNF-α active sites, while compound 22 showed potent inhibition of 5-lipoxygenase.[41] Molecular docking analysis also indicated that compound 2 binds specifically to the NLRP3 NACTH inhibitory domain, because compound 2 and MCC950 (control positive) bound at sites very close to this domain with a Gibbs free energy value equivalent to −10.6 kcal/mol and −9.7 kcal/mol, respectively.[92] Biflavonoid's anti-inflammatory activity is supported by its ability to interact with key inflammatory targets through molecular docking and its modulation of inflammatory pathways, making it a promising candidate for further research and development.

Conclusion

These findings suggest that the Araucariaceae family contains numerous biflavonoids, including amentoflavone, agathisflavone, cupressuflavone, robustaflavone, and their derivatives. According to this article, approximately 46 biflavonoids have been identified from the Araucariaceae family. However, their biological activities, particularly as anti-inflammatory agents, have yet to be fully explored, leaving many biflavonoids unexplored. Therefore, future research should be inspired to explore the relationship between their chemical composition and pharmacological effects based on geographical sources. Biflavonoids demonstrate significant potential as anti-inflammatory agents, capable of treating various inflammatory diseases. Initial studies indicate that biflavonoids employ multiple

mechanisms to combat inflammation, including the inhibition of pro-inflammatory enzymes. Further comprehensive investigations could reveal how biflavonoids from the Araucariaceae family suppress the expression of pro-inflammatory molecules. Given these unique properties, biflavonoids hold promise as anti-inflammatory drugs, particularly for managing chronic inflammatory diseases, inspiring further research in this field.

Conflict of Interest
The authors declare that there is no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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