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Justicia wynaadensis: A comprehensive review on pharmacological and molecular investigations of bioactive compounds with special emphasis on hypercholesterolemic potential

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Abstract
Cardiovascular disease (CVD) remains the leading cause of mortality worldwide, largely driven by hypercholesterolemia and associated metabolic disorders. In the search for sustainable alternatives to synthetic lipid-lowering agents, natural products and plant-derived bioactive compounds have emerged as a promising frontier due to their multifaceted pharmacological properties and reduced side effects. *Justicia wynaadensis* (locally known as "Karimkurinji"), a perennial shrub endemic to the Western Ghats of India, has drawn increasing scientific attention for its profound ethnopharmacological importance, nutritional value, and therapeutic potential. Traditionally consumed by tribal communities, particularly the Kodavas, during the monsoon season, the plant is valued for its rejuvenating, hepatoprotective, and detoxifying properties. Recent investigations highlight the plant's rich phytochemical profile, including flavonoids, phenolics, phytosterols, carotenoids, and natural pigments, which confer diverse pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, wound-healing, chemoprotective, and anticancer effects. Seminal studies on *J. wynaadensis* have identified key compounds such as 3,3′,4′-trihydroxyflavone and demonstrated significant bioactivity in both *in vitro* and *in vivo* models, although its direct impact on cholesterol metabolism remains comparatively underexplored. Given the confirmed presence of phytosterols and flavonoids, the plant exhibits strong potential as a natural hypocholesterolemic agent. This review synthesizes current knowledge on the ethnobotany, phytochemistry, pharmacology, and molecular mechanisms of *J. wynaadensis*, with a particular emphasis on its relevance to hypercholesterolemia. We highlight existing research gaps, propose mechanistic insights into lipid regulation based on its known constituents, and identify future directions, including omics-based approaches, molecular docking studies, and food-pharma applications. By consolidating traditional wisdom and modern pharmacological evidence, this work underscores the immense potential of *J. wynaadensis* as a functional food and a therapeutic candidate for managing cholesterol and associated metabolic disorders.

Keywords: *Justicia wynaadensis*, phytochemicals, hypercholesterolemia, bioactive compounds, pharmacology, flavonoids, phytosterols, cardiovascular health

1. Introduction

Hypercholesterolemia stands as a major modifiable risk factor for the development and progression of cardiovascular diseases (CVDs), contributing significantly to the global burden of atherosclerosis, myocardial infarction, and stroke (Li*et al.*, 2021) [28]. Conventional lipid-lowering therapies, with statins being the cornerstone, have proven effective in managing cholesterol levels but are not without limitations. A significant portion of patients experience adverse effects, including myotoxicity, hepatotoxicity, and general drug intolerance, which can lead to poor compliance and discontinuation of therapy (Gupta*et al.*, 2020) [16]. This clinical challenge has intensified the global search for safer, plant-derived bioactive compounds that can serve as either alternatives or complementary therapies for dyslipidemia. Medicinal plants, rich in a diverse array of secondary metabolites such as flavonoids, phenolics, and phytosterols, offer multifaceted therapeutic benefits that extend beyond simple lipid lowering to include the modulation of lipid metabolism, fortification of antioxidative defense systems, and potent anti-inflammatory action (Tiwari & Pandey, 2020) [52].

India, one of the twelve centres of genetic diversity in the world (Zeven and de Wet, 1982) [41], is rich in floristic wealth comparising 3,000-5,000 species, of which approximately 1000 species are used as a source of food. Areas in and around the Western Ghats are major biodiversity hot spots holding numerous plant species that offer food and possess medicinal values. Since ages the communities who live in these areas mostly depend on traditional foods for energy as well as medicine to cure the ailments. People here use some plants confined to particular location during specific season or special occasions. Consuming plants as a special food or dish have unique historical and traditional background (Greeshma and Sridhar, 2016) [15].

The Western Ghats of India, a mountain range running parallel to the western coast of the Indian peninsula, is recognized as one of the world's preeminent biodiversity hotspots and harbors an immense wealth of endemic species with documented ethnomedicinal value. Among this rich botanical diversity, *Justicia wynaadensis* (Nees) T. Anderson, a member of the Acanthaceae family, stands out due to its unique cultural, nutritional, and pharmacological relevance. Known locally by names such as "Karimkurinji" or "Maddhu thoppu," the plant is deeply integrated into the cultural and medicinal practices of indigenous communities, most notably the Kodava community in the Kodagu district of Karnataka. A cornerstone of their tradition is a monsoon health ritual involving the consumption of a curry prepared from its leaves, which is believed to detoxify the body, restore physiological balance, and enhance immunity (Deepthi*et al.*, 2024; Medappa S*et al.*, 2011) [9, 31].Over the past two decades, scientific exploration of *J. wynaadensis* has transitioned from documenting traditional knowledge to validating it through rigorous pharmacological investigation, uncovering a broad spectrum of bioactivities. Various extracts of the plant have been shown to possess potent antioxidant (Karunakaran*et al.*, 2022) [21], antimicrobial (Krishna*et al.*, 2021) [24], anticancer (Shenoy*et al.*, 2013; Vandana and Shanti, 2017) [44, 55], and chemoprotective (Subin & Shylesh, 2013) [49] properties. Furthermore, the plant is a source of valuable natural pigments, such as anthocyanins, which hold considerable potential for applications in the food and nutraceutical industries (Patil*et al.*, 2015) [34]. Despite this promising body of evidence, systematic studies focused on its direct effects on cholesterol regulation and lipid metabolism have remained limited. The plant's established phytochemical richness, particularly its significant content of flavonoids and cholesterol-lowering phytosterols, provides a strong scientific rationale for its potential in managing hypercholesterolemia. Foundational research has already demonstrated its ability to lower cellular cholesterol concentrations and, critically, inhibit the uptake of oxidized low-density lipoproteins (Ox-LDL) by macrophages, a key step in atherosclerotic plaque formation (Subbiah & Norman, 2002) [48].

This review aims to consolidate the existing ethnobotanical, phytochemical, and pharmacological evidence for *Justicia wynaadensis* while critically assessing its underexplored role in cholesterol regulation. By highlighting established molecular insights, potential food applications, and future research directions, this work seeks to provide a comprehensive platform for the continued therapeutic exploration of this valuable medicinal plant.

2. Ethnobotanical Significance and Cultural Heritage

2.1 Traditional Uses and Cultural Integration

The ethnobotanical importance of *Justicia wynaadensis* is intricately woven into the cultural fabric of the indigenous and rural communities inhabiting the Western Ghats. Its most well-documented and culturally significant use is among the Kodavas of Kodagu, Karnataka. They prepare and consume a traditional dish known as "Karimkurinji Curry" exclusively on the eighteenth day of the Kodava month of *Kakkada*, an event called *Kakkada Padinett*, which corresponds to the peak of the monsoon season (Deepthi*et al.*, 2024) [9]. This ritual is anchored in a profound belief that the plant, having absorbed the essence of the monsoon rains, possesses its most potent medicinal properties during this specific time. In local health traditions, it is considered a *Rasayana* (rejuvenator), believed to detoxify the bloodstream, fortify the immune system against monsoon-related ailments like fevers, and restore overall physiological balance. This traditional wisdom forms the very foundation for modern scientific inquiry into its properties (Karunakaran*et al.*, 2024) [22]. An interesting anecdotal observation from the local population is that upon ingestion of the plant's extract, a purple-colored urine is noticed, a curious biomarker that hints at the presence of unique, bioavailable metabolites (Subbiah & Norman, 2002) [48].

Beyond this specific ritual, the plant is valued as a seasonal functional food and a versatile folk medicine. Local healers and community elders have traditionally recommended its leaves for treating a wide range of conditions, including digestive disturbances, fevers, skin disorders, ulcers, leucorrhoea, and general debility (Karunakaran *et al.*, 2024; Deepthi *et al.*, 2024) [22, 9]. Furthermore, the Kurichiar tribes of the Wayanad district in Kerala, who call the plant "Kattu vatamkodi, crush the leaves and boil them in coconut oil to create a topical application used to treat rheumatic swellings (Udayan *et al.*, 2008) [53]. These diverse applications underscore its established role as a blood purifier, a general tonic for rejuvenation, and a cornerstone of regional ethnomedicine.

The most well-documented use of *J. wynaadensis* is among the Kodava tribes of the Kodagu district in Karnataka. Known to them as "Maddhu thoppu," the plant is integrated into their cultural and ritualistic functions. The community prepares a deep purple-colored extract from its leaves and stems, incorporating it into a sweet dish that is consumed as part of a traditional health practice. This ritual is believed to promote overall health and protect against diseases throughout the year. Beyond this general use, the plant is a staple in their folk medicine for treating common ailments like fever, digestive complications, skin disorders, and wounds. The integration of this plant into their daily life highlights how ethnobotanical practices are intertwined with the cultural identity of the Kodava people.

2.2 Influence of Seasonal and Lunar Cycles on Traditional Harvesting

A fascinating aspect of the ethnobotanical knowledge surrounding *J. wynaadensis* is the precise timing of its harvest, which is dictated by both seasonal and, remarkably, celestial cycles. The belief that its medicinal properties peak between June and August is a consistent theme in traditional practice (Subbiah & Norman, 2002) [48]. This period aligns with the *Kataka* or *Adi* month of the Hindu calendar, during which the plant is traditionally consumed (Vasundhara *et al.*, 2018) [54].

Adding another layer of complexity, recent scientific investigation has explored the influence of lunar cycles on the plant's phytochemistry, lending credence to these finely tuned traditional practices. A study by Vasundhara*et al.* (2018) [54] discovered that the anthocyanin content in the leaves was significantly higher on *Amavasya* (New Moon) days compared to *Pournima* (Full Moon) days. The peak accumulation of anthocyanin pigment (11.31 mg/L) was recorded on the New Moon day of early August, astonishingly aligning with the traditional Kodava harvesting period. The authors concluded that the lunar effect appears to be a predominant factor in the accumulation of these pigments, drawing parallels to similar findings in other medicinal plants like Ashwagandha, where lunar cycles are known to influence phytoconstituents. This suggests that traditional calendrical knowledge is not arbitrary but may be optimized to capture not just seasonal, but also celestial, influences on the plant's medicinal potency.

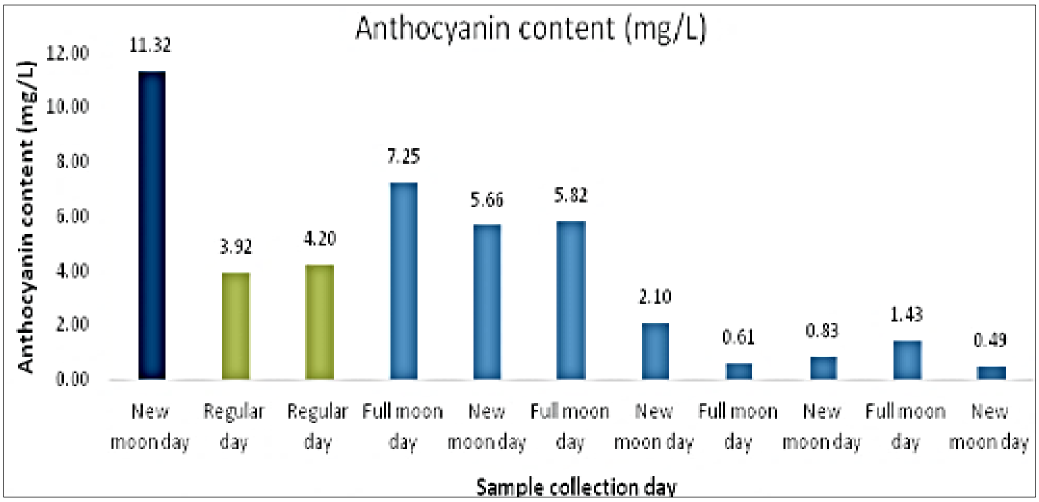

Fig 1: Influence of lunar cycles on anthocyanin content (mg/L) of justicia wynaadensis

2.3 Geographical Distribution and Conservation Status

Justicia wynaadensis is an endemic species confined to the unique ecological niches of the Western Ghats, with its presence documented across the states of Karnataka, Kerala, and parts of Tamil Nadu.*Justicia wynaadensis*, belonging to the Acanthaceae family, is reported to be endemic to the regions of Western Ghats, from South Canara, Coorg (Kodagu) to Wynaad, East Nilgiris and South Malabar Hills in South India, up to 3000 ft in evergreen forests and on waste lands. (Gamble, 1967) [40] It is a shade-loving understory shrub that thrives in the moist, humus-rich soils of tropical evergreen and semi-evergreen forests, often found in the understory (Krishnamurthy *et al.*, 2012) [25]. This specialized habitat, however, makes it particularly vulnerable to anthropogenic pressures.

Due to escalating threats of deforestation for agriculture and infrastructure, habitat fragmentation, and indiscriminate or over-harvesting, its natural populations are under considerable stress. The rising demand and growing recognition of its nutraceutical and medicinal value have paradoxically exacerbated these threats, placing immense pressure on wild populations. In response to this challenge, concerted conservation efforts have been initiated. A successful and critical intervention has been the development of a micropropagation protocol using nodal explants. This biotechnology-driven approach allows for the high-frequency regeneration and rapid, large-scale multiplication of the plant, ensuring a sustainable and consistent supply for both traditional communities and scientific research without depleting wild populations (Sullimada U P & Kiragandur M, 2012) [50]. Such initiatives, alongside the establishment of *ex situ* conservation plots, are vital not only for preserving the species' genetic diversity but also for safeguarding the traditional knowledge systems that rely on this plant, ensuring that its utilization involves equitable benefit-sharing with its indigenous custodians.

3. Botanical and Taxonomic Overview

3.1 Taxonomy and Classification

Justicia wynaadensis (Nees) T. Anderson is taxonomically situated within the Acanthaceae family, a large and diverse family of flowering plants comprising nearly 250 genera and more than 4,000 species, many of which are renowned for their medicinal importance (Daniel, 2016) [57]. It belongs to the genus *Justicia*, which is one of the largest within the family, with approximately 600 species widely distributed across tropical and subtropical ecosystems (Daniel, 2016; John*et al.*, 2014) [57, 20]. Justicia is the largest genus of Acanthaceae, with approximately 600 species that are found in pantropical and tropical regions. (Correa and Alcantara, 2012) [8]

Taxonomically, it is classified within the Kingdom Plantae and the Order Lamiales. Due to morphological similarities and overlaps with other congeneric species, accurate identification can be challenging but is crucial for both research and conservation purposes. Distinguishing botanical features include its characteristically lanceolate leaves with acuminate tips, unique venation patterns, and, most strikingly, the deep purple pigmentation that develops on its leaves and stems during the monsoon season (Karunakaran*et al.*, 2022) [21].

3.2 Botanical Description and Phenology

Morphologically, *J. wynaadensis* is a perennial scandent herb or shrub that typically grows to a height of 30-60 cm. It features slender, erect, and slightly branched stems. The leaves are simple, opposite, and lanceolate with entire margins, measuring between 5 and 15 cm in length. A remarkable characteristic of the plant is the seasonal change in its appearance; during the rainy season, the leaves exhibit a distinct purplish tinge due to the accumulation of anthocyanin pigments. Its flowers are small, tubular, and violet-blue with a bilabiate corolla, arranged in short, terminal spikes. The fruit is a small, dehiscent capsule containing seeds, which is characteristic of the Acanthaceae family (Krishnamurthy *et al.*, 2012) [25].

The plant exhibits remarkable phenological plasticity, particularly in its phytochemical expression, which provides a strong scientific rationale for the timing of its traditional harvest. The accumulation of anthocyanin pigments during the monsoon is understood to be a physiological response to abiotic stressors such as high rainfall and altered light intensity, likely serving to protect the plant's photosynthetic machinery. Crucially, scientific studies have confirmed that this seasonal change is not merely aesthetic. Multiple investigations have demonstrated that the concentration of key bioactive compounds, including total phenolics and flavonoids, and consequently, the plant's overall antioxidant capacity, peaks during the monsoon period (Deepthi*et al.*, 2024; Karunakaran*et al.*, 2022) [9, 21]. This empirical evidence directly validates traditional harvesting practices and provides a scientific basis for the ethnomedicinal timing of its consumption.

4. Nutritional and Phytochemical Profile

4.1 Nutritional Value

Consumed as a functional seasonal vegetable, the leaves of *J. wynaadensis* offer a compelling nutritional profile that underpins its role as a health-promoting food. Proximate composition analyses have confirmed its status as a good source of dietary fiber, proteins, and essential minerals (Deepthi*et al.*, 2024) [9]. It is particularly rich in macro- and micro-minerals, including calcium, magnesium, iron, potassium, and zinc. Studies on mineral content have shown that while many minerals are highest at the onset of the monsoon, key nutrients like potassium remain high into the peak medicinal period, making the plant a valuable dietary supplement for regulating body fluids and combating mineral-deficiency-related abnormalities (Karunakaran*et al.*, 2022) [21]. The plant also contains notable levels of vitamins, particularly vitamin C (ascorbic acid) and the provitamin A carotenoid, β-carotene (Deepthi*et al.*, 2024) [9]. The nutritional value of *J. wynaadensis* is substantially enhanced by its exceptionally high content of polyphenols and anthocyanins, which confer potent antioxidant properties. When compared to conventional leafy greens, *J. wynaadensis* demonstrates superior levels of total phenolics and flavonoids, especially during its monsoon growth phase, positioning it as a nutritionally dense and medicinally valuable dietary herb (Deepthi*et al.*, 2024) [9]. The phytochemical studies were conducted on various parts (stem, leaf, and root) of seven Justicia species, such as *J. wynaadensis*, *J. adhatoda*, and *J. beddomei*. The research revealed that the various plant parts exhibited striking variations in their chemical composition. The HPTLC profile developed serves as a convenient analytical tool for the quality determination of these medicinal herbs. (Sini and George, 2020) [47].

4.2 Phytochemical Constituents

Various species of Justicia have been used variously for treating a variety of ailments by various tribal people. Based on the strong evidence of biological activities of phenolic compounds, the study was focused on determination of total phenolics and Flavonoids in different parts of selected Justicia species such as J. adhatoda, J. beddomei, J. betonica, J. gendarussa, J. montana and J. wynaadensis. The results showed that the leaf extract of J. beddomi showed highest alkaloid content (28.53mg CE) followed by leaf extract of J. wynaadensis (26.96 mg CE) and J. betonica (26.18 mg CE). The root of J. wynaadensis showed least alkaloid content (8.45 mg CE). The higher alkaloid content was revealed in the leaves (John*et al.*, 2014) [57].

The therapeutic potential of *J. wynaadensis* is rooted in its complex and diverse phytochemical composition. Methodical investigations using advanced chromatographic and spectroscopic techniques such as High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), and Gas Chromatography-Mass Spectrometry (GC-MS) have elucidated a wide array of bioactive compounds. The most prominent classes include flavonoids, phenolic acids, phytosterols, alkaloids, fatty acids, terpenoids, saponins, tannins, and natural pigments like anthocyanins and carotenoids (Karunakaran*et al.*, 2022; Krishna*et al.*, 2021) [21, 24].

Flavonoids: The flavonoid profile is particularly notable, with 3,3′,4′-Trihydroxyflavone (THF) being identified as a key bioactive marker responsible for many of its pharmacological effects, including profound wound-healing and antimicrobial properties (Krishna*et al.*, 2021; Dsouza & Nanjaiah, 2017) [24, 12]. This compound was successfully isolated from the most potent chloroform fraction using bioassay-guided fractionation and its structure was elucidated using NMR and LC-MS (Dsouza & Nanjaiah, 2017) [12]. The flavonoid profile is further enriched by derivatives of other important flavones like luteolin, apigenin, and quercetin glycosides.

Phenolic Acids: The plant is a rich source of various phenolic acids which contribute significantly to its free-radical scavenging and anti-inflammatory activities (Karunakaran*et al.*, 2022) [21]. A detailed analysis by Karunakaran*et al.* (2022) [21] identified and quantified 14 distinct phenolic acids, revealing a dynamic metabolic profile that shifts with the seasons. Among these, salicylic acid was found to be the most abundant, with its concentration increasing by 43.8% during the peak monsoon period. This peak aligns with the plant's traditional use for skin ailments, as salicylic acid is a well-established keratolytic and bacteriostatic agent. Other crucial hydroxycinnamic acids that peak during this time include caffeic acid (increasing by 126.9%) and o-coumaric acid (increasing by 70.3%), while their precursors decrease, suggesting an accelerated biosynthesis of these medicinal end-products. The profile also includes gallic acid, ferulic acid, p-coumaric acid, and protocatechuic acid, among others.

Phytosterols: Of critical relevance to its hypocholesterolemic potential is the presence of a significant concentration of phytosterols. GC-MS analysis has confirmed the presence of β-sitosterol, stigmasterol, and campesterol (Patil*et al.*, 2015; Ponnamma and Manjunath, 2012) [34, 36]. These plant sterols are structurally analogous to cholesterol, distinguished by minor modifications on their side chains, and are well-known to directly interfere with intestinal cholesterol absorption, providing a strong mechanistic basis for the plant's traditional use in cholesterol reduction.

Alkaloids: The *Justicia* genus is known to be rich in alkaloids, and *J. wynaadensis* is no exception. A comparative spectrophotometric study by John*et al.* (2014) [20] found that its leaves possess a high alkaloid content (26.96 mg CE), second only to *J. beddomei* among the six species tested. In contrast, the root showed the least alkaloid content (8.45 mg CE), confirming that the leaves are the primary repository for these bioactive compounds.

Fatty Acids and Terpenoids: Bioassay-guided screening has also identified several fatty acids and terpenoids as key contributors to the plant's antimicrobial activity. GC-MS analysis of active fractions revealed the presence of the diterpene phytol, the terpenoid neophytadiene, and a suite of fatty acids including myristic acid, palmitic acid, stearic acid, linoleic acid, and methyl linoleate (Ponnamma & Manjunath, 2015) [37]. These compounds, particularly phytol and the free fatty acids, are known to exert antimicrobial effects by disrupting bacterial cell membranes.

Pigments (Anthocyanins and Carotenoids): The vibrant purple pigmentation observed during the monsoon is attributed to water-soluble anthocyanin pigments, primarily cyanidin-3-glucoside. These pigments are not merely for coloration but are potent antioxidants with their own health-promoting properties. Additionally, the plant contains carotenoids such as lutein and β-carotene, which further enhance its antioxidant profile and nutritional value (Patil*et al.*, 2015) [34]. The seasonal variability in the concentration of these phytochemicals provides a robust scientific rationale for the specific timing of traditional harvesting for both culinary and medicinal applications.

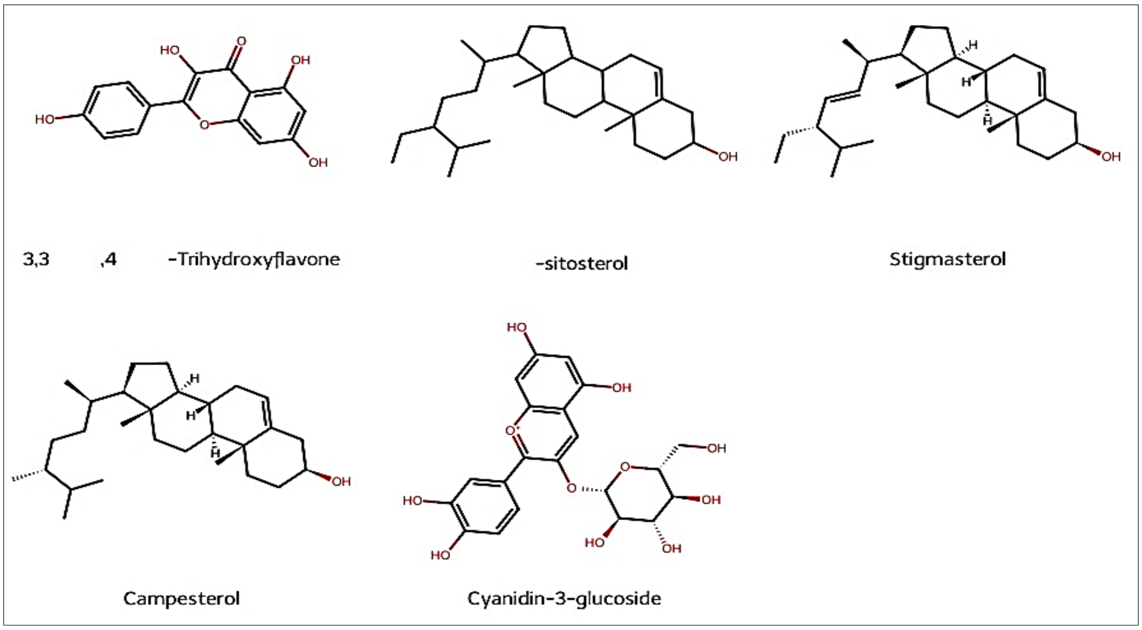

Fig 1: Chemical Structures of Identified Compounds

5. Validated Pharmacological Activities

Across included records, *J. wynaadensis* consistently demonstrates season-peaking antioxidant capacity, bioassay-guided antimicrobial and wound-healing activity centered on 3,3′,4′-trihydroxyflavone, chemoprotection against cyclophosphamide, preliminary cytotoxicity in select cancer lines, and a patent reporting cellular cholesterol reduction with inhibition of Ox-LDL uptake; however, no animal lipid-profile studies were identified, constituting the central evidence gap for hypercholesterolemia claims. The rich and diverse phytochemistry of *J. wynaadensis* translates into a broad array of pharmacological activities, many of which have been rigorously validated through modern scientific methods, corroborating its traditional therapeutic claims and suggesting novel applications.

5.1 Antioxidant and Chemoprotective Activity

The antioxidant activity of *J. wynaadensis* is perhaps its most well-characterized property, underpinning many of its other therapeutic effects. Methanolic leaf extracts exhibit a high total phenolic and flavonoid content, which correlates directly with robust radical scavenging capabilities in various chemical assays, including the decolorization of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the quenching of the radical cation in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (Karunakaran*et al.*, 2022) [21]. This antioxidant capacity peaks during the monsoon season, coinciding with maximum anthocyanin accumulation, which suggests that its traditional consumption may help mitigate oxidative stress—a key pathogenic process implicated in CVD, cancer, and aging (Deepthi*et al.*, 2024) [9]. This potent antioxidant power extends to a significant chemoprotective effect. An important *in vivo* study in Swiss albino mice investigated the plant's ability to protect against damage induced by the chemotherapy drug cyclophosphamide. Pre-treatment with an aqueous extract of *J. wynaadensis* was found to significantly mitigate the genotoxicity and oxidative stress caused by the drug, suggesting a potential role as an adjuvant therapy in chemotherapy to reduce drug-induced DNA damage and other side effects (Subin & Shylesh, 2013; B.L., Vishma *et al*.) [49].

5.2 Antimicrobial and Wound Healing Activity

J. wynaadensis has demonstrated significant and broad-spectrum antimicrobial effects. One of the most significant discoveries in this area was the identification of 3,3′,4′-trihydroxyflavone (THF) as a major antimicrobial agent through bioassay-guided fractionation (Krishna*et al*., 2021) [24]. This flavonoid exhibit powerful activity against a range of clinically relevant pathogens, including the Gram-positive bacterium Staphylococcus aureus, the Gram-negative bacterium Escherichia coli (both common in diabetic wound and urinary tract infections), and the opportunistic fungus Candida albicans (Dsouza, D. and Nanjaiah, 2018; Krishna*et al*., 2021) [13, 24]. Other constituents, including the terpenoid phytol and various fatty acids, have also been identified as being responsible for inhibitory action against pathogens like Klebsiella pneumoniae (Ponnamma and Manjunath, 2015) [37]. This activity supports its ethnomedicinal use as a digestive and detoxifying herb and suggests its potential for developing novel antimicrobial agents.

The plant's most remarkable efficacy has been demonstrated in wound healing, particularly in compromised conditions like diabetes. The therapeutic potential of the isolated flavonoid, THF, was brilliantly demonstrated in a streptozotocin-induced diabetic rat excision wound model (Dsouza & Nanjaiah, 2017) [12]. Topical application of a THF-containing cream resulted in a significant, dose-dependent acceleration of wound contraction and closure. On day 14 of the study, the higher dose (50 mg/kg BW) of THF cream achieved 61% wound closure, outperforming the standard commercial antibiotic cream, framycetin (56%). By day 28, the THF-treated groups showed complete wound healing, whereas the untreated diabetic control group showed less than 50% closure. Histopathological examination confirmed these visual findings, revealing that THF treatment promoted the restoration of normal skin architecture with robust collagen deposition, stimulated the proliferation of fibroblasts and capillaries, and increased re-epithelialization. This potent effect is attributed to a combination of its direct antimicrobial action, which reduces the inflammatory phase, and its ability to stimulate collagen synthesis and angiogenesis (Krishna*et al*., 2021; Dsouza & Nanjaiah, 2017) [24, 12].

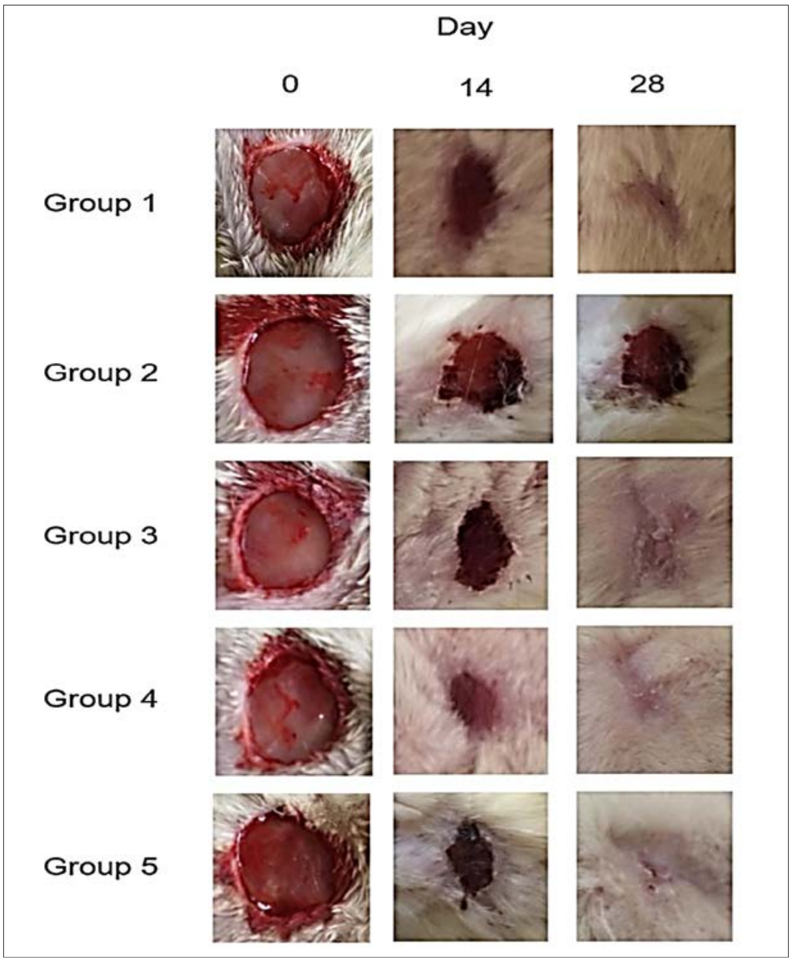

Fig 2. 3,3',4'-Trihydroxyflavone showed higher contraction rate and accelerated the healing process. Group1, normal control rats showed complete healing after 28 days. Group2, diabetic untreated rats showing no significant improvement during the experimental period. Group3 and 4 rats treated with 25 and 50 mg/kg BW of 3,3',4'-Trihydroxyflavone, respectively showed significant contraction and healing of the wound. Group 5 rats treated with standard drug, framycetin with complete wound healing.

5.3 Anticancer and Antimitotic Activity

In the realm of oncology, *J. wynaadensis* has demonstrated promising anticancer effects in preliminary screenings. *In vitro* studies have shown that its extracts induce cytotoxicity against human breast (MCF-7) and colon (HCT-116) cancer cell lines (Shenoy*et al*., 2013; Vandana and Shanti, 2017) [44, 55]. The cold aqueous extract was found to be the most potent, showing low IC_{50} values and marking it as a promising candidate for further anticancer drug development (Vandana and Shanti, 2017) [55]. The proposed mechanism of action involves the induction of apoptosis (programmed cell death), which is characterized by the disruption of mitochondrial membrane potential and the activation of caspase cascades (Shenoy*et al.*, 2013) [44]. Furthering this line of research, nanoparticles synthesized using the plant's extract have shown novel mechanisms of anticancer action. Zinc Oxide Nanoparticles (ZnO-NPs) green-synthesized with the extract exhibited strong antimitotic (cell division-inhibiting) activity and DNA-binding capabilities, highlighting a potential new pathway for its anticancer effects that warrants further investigation (Kumar*et al.*, 2019) [26]. These initial *in vitro* findings provide a strong basis for future validation in animal models.

5.4 Antidiabetic and Anti-inflammatory Potential

Preliminary studies have indicated potential antidiabetic activity for *J. wynaadensis*. The plant's rich flavonoid content, including compounds like THF, is hypothesized to inhibit key carbohydrate-metabolizing enzymes such as α-amylase and α-glucosidase. By slowing the breakdown and absorption of complex carbohydrates in the digestive tract, these compounds can help reduce postprandial hyperglycemia, which is a key therapeutic target in managing type 2 diabetes and the broader metabolic syndrome. Although promising, this area requires more direct investigation and larger-scale trials for confirmation.The plant also possesses documented anti-inflammatory properties, which are comparable to the standard drug diclofenac and are attributed to the synergistic action of its alkaloids, steroids, flavonoids, and cardiac glycosides (Vidyabharathi). This activity is a crucial component of its effectiveness in wound healing and provides further rationale for its traditional use in treating inflammatory conditions like rheumatic swelling.

6. Hypercholesterolemic Potential: A Mechanistic Review

While direct clinical studies on the cholesterol-lowering activity of *J. wynaadensis* are limited, its well-documented phytochemical profile provides a strong mechanistic basis for its potential in managing hypercholesterolemia, a primary driver of atherosclerosis and CVD. The foundational evidence stems from a seminal patent by Subbiah & Norman (2002) [48], which made two groundbreaking claims: first, that the plant extract effectively lowers cellular cholesterol and cholesteryl ester concentrations, and second, that it exhibits a novel inhibitory effect on the uptake of oxidized low-density lipoproteins (Ox-LDL) by human macrophage cell lines. The uptake of Ox-LDL by macrophages is the critical initiating step in the formation of foam cells, the hallmark of atherosclerotic plaques. By inhibiting this fundamental process, the extract directly targets the pathogenesis of cardiovascular disease. This potential is mediated through the synergistic action of its key bioactive compounds.

6.1 Flavonoids and Phytosterols: A Two-Pronged Attack on Cholesterol

The flavonoids present in *J. wynaadensis*, such as THF, luteolin, and apigenin, are expected to modulate lipid metabolism through systemic mechanisms. Structurally similar flavonoids are known to inhibit HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway of cholesterol biosynthesis, which is the same molecular target of statin drugs (Tiwari & Pandey, 2020) [52]. Furthermore, these compounds may act as agonists for peroxisome proliferator-activated receptors (PPARs), particularly PPAR-α. These nuclear receptors are master regulators of lipid metabolism, and their activation promotes fatty acid β-oxidation and increases the synthesis of cardioprotective high-density lipoprotein (HDL) cholesterol (Tiwari & Pandey, 2020) [52]. However, the most direct and potent hypocholesterolemic mechanism likely stems from the plant's high content of phytosterols, namely β-sitosterol, stigmasterol, and campesterol. The process of dietary cholesterol absorption begins with its incorporation into bile salt micelles within the intestinal lumen. These micelles are then transported to the brush border membrane of enterocytes for uptake, a process primarily mediated by the Niemann-Pick C1-Like 1 (NPC1L1) transporter. Due to their structural similarity to cholesterol, phytosterols effectively compete for limited space within these micelles. This competitive displacement reduces the amount of cholesterol that can be solubilized and made available for absorption, leading to its increased excretion in feces (Patil*et al.*, 2015) [34]. In addition to this competitive inhibition, phytosterols may also downregulate the expression of the NPC1L1 transporter itself, further limiting cholesterol uptake, and upregulate hepatic LDL receptors to enhance the clearance of cholesterol from the bloodstream.

6.2 Anthocyanins and Synergistic Poly-pharmacology

The anthocyanins, such as cyanidin-3-glucoside, which are responsible for the seasonal purple hue of the leaves, contribute a further layer of cardiovascular protection. Beyond their potent antioxidant effects that prevent the oxidation of LDL a critical step in atherogenesis these pigments have been shown to modulate the expression of key genes involved in lipid transport and metabolism (Li*et al.*, 2021) [28]. For instance, they can upregulate the expression of ATP-binding cassette transporter A1 (ABCA1), a crucial protein that mediates reverse cholesterol transport, the process of removing excess cholesterol from peripheral tissues (like macrophages in the artery wall) and transporting it back to the liver for excretion.

The true therapeutic strength of J. wynaadensis likely lies in this synergistic poly-pharmacology. It represents a multi-target approach that is a hallmark of many effective herbal medicines. While phytosterols work locally to block cholesterol absorption at the intestinal level, flavonoids and anthocyanins work systemically to reduce endogenous cholesterol synthesis, prevent LDL oxidation, reduce inflammation, improve endothelial function, and enhance reverse cholesterol transport. Despite this strong phytochemical and mechanistic evidence, a significant research gap remains, as there is a lack of dedicated *in vivo* studies using animal models of hypercholesterolemia to confirm and quantify the effects of J. wynaadensis extracts on lipid profiles (TC, LDL-C, HDL-C).

7. Seasonal Variation in Phytochemicals and Minerals of Justicia wynaadensis

In the Western Ghats, local communities traditionally consume Justicia wynaadensis in early August, believing this period enhances its wound-healing and curative properties. To validate these practices, a study was conducted to examine temporal changes in phenolics, flavonoids, carotenoids, and mineral constituents across different monsoon harvesting stages. Alam*et al*. (2016) [1] reviewed the biochemical mechanism of antiinflammatory effects of hydroxyl cinnamic acids, ferulic acid, pcoumaric acid, caffeic acid, and chlorogenic acid, and their derivatives. Sinapic acid, with reported antioxidant and antiinflammatory properties, is also present in fruits and vegetables (Silambarasan*et al*., 2016) [46]; it is biosynthesized from caffeic acid, through intermediate ferulic acid.

7.1 Phenolic and Flavonoid Dynamics

Total phenolic content (TPC) varied significantly, ranging from 323.8 mg GAE 100 g^{-1} at the onset of monsoon (AS-I) to 388.2 mg GAE 100 g^{-1} in mid-monsoon (AS-II), representing a 20 % increase. By the late monsoon stage (AS-III), TPC declined by about 6 %. Flavonoid content exhibited a similar trend, increasing from 138 mg CE 100 g^{-1} at AS-I to 287 mg CE 100 g^{-1} at AS-II a 108.4 % rise followed by a 33 % decrease post-monsoon. This mid-monsoon enrichment in phenolics and flavonoids supports their known roles in mitigating oxidative stress and chronic diseases such as cardiovascular disorders, diabetes, and cancer, as highlighted by Pandey and Rizvi (2009) [60].

7.2 Antioxidant Activity and Carotenoid Content

Although antioxidant activity, measured through FRAP (3.11-3.35 mg AEAC 100 g^{-1}) and DPPH (103.56-133.91 mg AEAC 100 g^{-1}) assays, did not display a clear seasonal pattern, values confirmed the plant's functional food potential. Carotenoids peaked during AS-II at 2.40 µg β-CE 100 g^{-1}, a 42 % rise over AS-I, before falling sharply (80 %) in AS-III. Carotenoids, including β-carotene, are precursors of vitamin A and are critical for skin health, immunity, and vision (Nkondjock and Ghadirian 2004) [33]. Chlorophyll a, chlorophyll b, and total chlorophyll declined steadily from AS-I to AS-III, despite chlorophyll's documented anti-inflammatory, antioxidant, anticancer, and wound-healing properties.

7.3 Phenolic Acid Profiles and Bioactive Compounds

Fourteen phenolic acids were identified, including salicylic, caffeic, ferulic, and o-coumaric acids. Salicylic acid was the most abundant, ranging from 2455 to 4747 µg g^{-1}. Its concentration increased by 43.8 % in AS-II compared to AS-I and then decreased by 48.3 % in AS-III. Elevated salicylic acid levels in mid-monsoon coincide with heavy rainfall and optimal humidity, conditions that may enhance biosynthesis. Salicylic acid is widely recognized for its keratolytic, bacteriostatic, and comedolytic properties and is used in treating warts, acne, dandruff, psoriasis, and hyperpigmentation (Arif 2015 [2]; Bikowski 2004 [3]; Saoji and Madke 2021 [42]; Madan and Levitt 2014 [30]; Bosund*et al.* 1960 [4]; Kornhauser*et al.* 2012) [23]. Its presence at higher concentrations in August likely contributes to the plant's reputed curative effects.

Caffeic acid and o-coumaric acid increased markedly at AS-II, by 126.9 % and 70.3 %, respectively, before declining in AS-III. Ferulic acid levels remained stable through AS-II but dropped later in the season. These hydroxycinnamic acids are associated with antioxidant, antimicrobial, UV-protective, and anti-inflammatory activities (Espínola*et al.* 2019 [14]; Taofiq*et al.* 2017 [51]; Silva*et al.* 2014 [45]; Rodrigues*et al.* 2015 [39]; Silambarasan*et al.* 2016) [46]. Gentisic acid doubled in AS-II, whereas gallic acid and other hydroxybenzoic acids (p-hydroxybenzoic, protocatechuic, ellagic, vanillic, and 2,4-dihydroxybenzoic acids) decreased. Such variations suggest shifts in phenylpropanoid metabolism, aligning with findings that salicylic acid synthesis in plants often proceeds via benzoate or o-coumarate intermediates (Chen*et al.* 2009) [7].

7.4 Mineral Composition Across Harvest Stages

Mineral profiling revealed significant seasonal differences. Calcium content was highest in AS-I (442 mg 100 g^{-1}) and decreased thereafter, while phosphorus peaked in AS-III (42.5 mg 100 g^{-1}). Iron content was greatest at AS-I (3.13 mg 100 g^{-1}) but declined in later stages. Zinc showed the opposite trend, increasing toward AS-III. Potassium levels remained comparatively stable between AS-I and AS-II. The presence of essential minerals such as potassium, calcium, and iron suggest that *J. wynaadensis* can act as a valuable dietary supplement.

7.5 Principal Component Analysis and Implications

Principal component analysis (PCA) explained 63.07 % of the variance in PC1 and 36.93 % in PC2. AS-II samples clustered with variables such as TPC, total flavonoids, carotenoids, salicylic acid, caffeic acid, and o-coumaric acid, confirming mid-monsoon as the optimal harvest stage for bioactive enrichment. AS-I grouped with chlorophylls, ellagic acid, gallic acid, vanillic acid, protocatechuic acid, and minerals like calcium, potassium, and iron, while AS-III aligned with later-season compounds.The findings clearly demonstrate that *J. wynaadensis* leaves collected in early August (AS-II) contain the highest levels of phenolics, flavonoids, carotenoids, and salicylic acid, aligning with indigenous knowledge that identifies this period as the most medicinally potent. This seasonal enrichment in phytochemicals likely underpins the plant's curative reputation and strengthens its potential as a functional food and therapeutic resource.

8. Novel Applications and Future Directions

Beyond its direct therapeutic potential, the unique properties of *J. wynaadensis* open avenues for novel applications in the food, nutraceutical, and biotechnology sectors.

8.1 Natural Pigments for Food and Nutraceutical Applications

The vibrant bluish-purple anthocyanin pigments in *J. wynaadensis*, particularly cyanidin-3-glucoside, represent a significant opportunity at the intersection of food science and pharmaceuticals. With growing regulatory scrutiny and consumer aversion to synthetic food dyes, the global demand for safe, natural, and functional alternatives has surged (Downham & Collins, 2000) [11]. A study by Patil*et al.* (2015) [34] successfully optimized the extraction of this pigment and characterized its stability, finding it to be stable in a neutral to slightly alkaline pH range (6.0-8.0) and at temperatures up to 50°C. These properties make it a viable and safe candidate for use as a natural food coloring in a variety of products, including beverages, yogurts, jams, confectionery, and baked goods.

The application of these pigments, however, extends beyond simple coloration into the realm of functional foods and nutraceuticals. As anthocyanins are recognized for their broad health-promoting properties, including anti-obesity, cardiovascular, and antidiabetic effects, an extract from *J. wynaadensis* could be developed into a dual-purpose food additive, providing both natural color and therapeutic benefits. Realizing this potential will require overcoming technical challenges. Optimized and green extraction techniques, such as ultrasound- or microwave-assisted methods, will be necessary to maximize yield while preserving bioactivity (Castañeda-Ovando*et al.*, 2009) [5]. Furthermore, stabilization technologies like microencapsulation will be crucial to protect the pigments from degradation due to pH, light, and temperature, thereby ensuring their efficacy and extending shelf life in commercial products.

8.2 Pioneering Applications in Nanotechnology

The plant's extract has proven to be an excellent reducing and capping agent for the green synthesis of nanoparticles, opening up novel therapeutic possibilities. Silver Nanoparticles (AgNPs) synthesized using the extract showed enhanced anticancer, antibacterial, and anti-inflammatory activities compared to the extract alone, Likewise AgNPs also showed strong antibacterial activity against various human pathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. (Lava*et al.*, 2021) [27]. In another pioneering study, Zinc Oxide Nanoparticles (ZnO-NPs) synthesized using the extract exhibited strong antimitotic and DNA-binding capabilities, suggesting a unique mechanism for its potential anticancer effects (Kumar*et al.*, 2019) [26]. These nanotechnology applications represent an exciting frontier for augmenting the plant's inherent bioactivities.

8.3 Future Research Perspectives

While the existing literature provides a robust foundation, it clearly points towards significant research gaps that must be addressed to fully harness the therapeutic potential of *J. wynaadensis*. A structured and multi-disciplinary research agenda is imperative. Future research must prioritize:

- **Pre-clinical *In vivo* Validation:** Conducting well-designed studies using established animal models of hypercholesterolemia (e.g., high-fat diet-fed rats or zebrafish models) is the most critical next step to empirically validate and quantify the plant's effect on lipid profiles and atherosclerotic plaque development.
- **Mechanistic *In vitro* Studies:** Utilizing relevant cell lines (e.g., human hepatoma HepG2, intestinal Caco-2) to elucidate precise molecular mechanisms, such as the direct measurement of HMG-CoA reductase inhibition, quantification of NPC1L1 expression, and analysis of SREBP-2 pathway activation.
- **Bioassay-Guided Fractionation:** Systematically isolating and identifying the specific compound(s) or fractions with the most potent hypocholesterolemic activity to move beyond crude extracts and towards standardized formulations.
- **Pharmacokinetic and Toxicological Profiling:** Conducting studies to determine the bioavailability, metabolism, and safety profile of the key bioactive compounds to establish a therapeutic window and ensure safety for human consumption.
- **Standardized Formulation and Clinical Trials:** Developing a standardized, quality-controlled extract and proceeding with randomized, placebo-controlled clinical trials, potentially beginning with pilot studies within the Kodava community, to definitively assess its efficacy and safety in human subjects.

9. Conclusion

Justicia wynaadensis stands as a powerful exemplar of how traditional ethnobotanical knowledge can guide modern scientific discovery. The current body of research confirms it is a reservoir of diverse bioactive compounds with a remarkable range of validated pharmacological activities, including potent antioxidant, antimicrobial, anti-inflammatory, chemoprotective, and wound-healing effects. While its traditional use as a general health tonic is now supported by robust scientific data, its most compelling and underexplored therapeutic frontier lies in the management of metabolic disorders, particularly hypercholesterolemia. The synergistic confluence of cholesterol-competing phytosterols, HMG-CoA reductase-inhibiting flavonoids, and gene-regulating anthocyanins provides a formidable scientific rationale for its lipid-lowering efficacy.Bridging the critical gap between its phytochemical promise and empirical, clinical validation is essential. By systematically pursuing the outlined research avenues from pre-clinical validation and mechanistic studies to eventual clinical trials the scientific community can fully unlock the therapeutic potential of *Justicia wynaadensis*. This endeavor holds the promise of developing this seasonal folk remedy into a globally recognized functional food or a source of novel, safe, and effective therapies in the crucial fight against cardiovascular disease.Justicia wynaadensis emerges as a unique convergence of traditional wisdom and modern pharmacological promise. Anchored in centuries of indigenous use, this lesser-known medicinal plant of the Western Ghats harbors a rich repertoire of bioactive compounds flavonoids, phytosterols, phenolic acids, anthocyanins, and carotenoids that collectively contribute to its broad-spectrum therapeutic potential. Scientific investigations have validated its antioxidant, anti-inflammatory, antimicrobial, wound-healing, chemoprotective, and anticancer properties, positioning it as a versatile candidate in ethnopharmacology. Most notably, its unexplored yet mechanistically plausible role in regulating cholesterol metabolism places *J. wynaadensis* at the frontier of natural interventions for cardiovascular health. Through a multi-targeted approach phytosterols reducing dietary cholesterol absorption, flavonoids modulating HMG-CoA reductase and PPAR pathways, and anthocyanins enhancing reverse cholesterol transport and preventing LDL oxidation the plant presents a comprehensive strategy against hypercholesterolemia and atherosclerosis. Despite this promise, critical gaps remain in translating phytochemical and *in vitro* insights into clinical relevance. Rigorous pre-clinical investigations, bioassay-guided fractionation, pharmacokinetics, toxicity studies, and standardized formulations are essential before its integration into functional food, nutraceutical, or therapeutic applications. The integration of omics-based tools, molecular docking, and nanotechnology-driven delivery systems could further unlock novel utilities. By bridging indigenous knowledge with cutting-edge pharmacological science, J. wynaadensis has the potential to evolve from a seasonal folk remedy into a globally relevant intervention for metabolic and cardiovascular disorders. Its journey from culturally revered "Karimkurinji" to a scientifically validated hypocholesterolemic agent exemplifies the immense opportunities that lie at the interface of biodiversity conservation, traditional medicine, and modern healthcare innovation.

Table 1: Evidence map of pharmacological activities

Study	Plant material (part; extract/fraction)	Analytical markers/methods	Model/system	Dose or concentration	Assays/ endpoints	Key results	Limitations
Karunakaran*et al.*, 2022 [21]	Leaves; methanol/water extracts	TPC/TFC; carotenoids; minerals by HPLC/UV; seasonal tracking	*In vitro* chemical assays	N/A	DPPH, ABTS, FRAP; seasonal profiles	Antioxidant capacity peaks during monsoon; phenolics/flavonoids increase with rainfall-linked phenology	*In vitro* only; no *in vivo* redox biomarkers
Deepthi*et al.*, 2024 [9]	Leaves; culinary preparation context	Nutritional panel; vitamin C; β-carotene; polyphenols	*In vitro* analyses	N/A	Proximate, minerals, vitamins	High polyphenols and nutrients, supporting functional food relevance	No intervention trial; seasonal intake patterns observational
Krishna*et al.*, 2021 [24]	Leaves; chloroform fraction; bioassay guided	3,3′,4′-trihydroxyflavone (THF) via NMR/LC-MS	Bacterial/fungal panels	MIC 32 µg/mL-1.2 mg/mL	MIC, inhibition zones vs clinical pathogens	THF shows broad antimicrobial activity (Gram±; UTI and diabetic wound isolates)	No *in vivo* infection model; stability not assessed
Dsouza & Nanjaiah, 2017 [12]	Isolated THF; topical cream	THF purity by spectroscopy	STZ-diabetic rat excision wound	25-50 mg/kg BW topical eq.	Wound contraction; histology; collagenization	Faster closure vs standard; complete healing by day 28 with THF	Single species; no systemic PK; dosing translation uncertain
Subin & Shylesh, 2013 [49]; B.L. Vishma*et al.*, 2017 [49]	Aqueous extract	Noted phytochemical classes	Swiss albino mice with cyclophosphamide	Extract pre-treatment	Genotoxicity assays; oxidative markers	Reduced oxidative stress; chemoprotective effect	Mechanism unresolved; no dose-response PK
Shenoy*et al.*, 2013 [44]; Vandana & Shanti, 2017 [55]	Aqueous/ethanolic extracts	Crude profiles	MCF-7, HCT-116 cell lines	IC50 values reported	Cytotoxicity; apoptosis markers	Induces apoptosis and cytotoxicity *in vitro*	Selectivity vs normal cells not defined
Kumar*et al.*, 2019 [26]; Lava*et al.*, 2021 [27]	Extract-synthesized ZnO-NPs/AgNPs	NP characterization (size, zeta)	Antimitotic; antibacterial models	NP doses per study	DNA binding; antimicrobial assays	Enhanced anticancer/antibacterial vs extract alone	Safety and reproducibility gaps; scale-up not addressed
Subbiah & Norman, 2002 (patent) [48]	Crude extract	N/A	Human macrophage cell lines	Extract concentrations	Cellular cholesterol; Ox-LDL uptake	Lowered cellular cholesterol and inhibited Ox-LDL uptake	Patent, not peer-reviewed; no animal lipid outcomes

Table 2: Quantified constituents and analytical methods

Class	Compound(s)	Quantification (method; units)	Seasonal/lunar variability	Relevance to lipid metabolism	Sources
Phenolic acids	Salicylic acid; caffeic; o-/p-coumaric; gallic; ferulic; protocatechuic	HPLC/UPLC-MS; mg/g DW; 14 acids profiled	Monsoon increases; salicylic +43.8%, caffeic +126.9%, o-coumaric +70.3% across season tracking	Antioxidant/anti-inflammatory support reducing LDL oxidation	Karunakaran 2022 [21]
Flavonoids	3,3′,4′-trihydroxyflavone (THF); luteolin/apigenin/quercetin glycosides	HPTLC/HPLC; NMR/LC-MS confirmation; mg/g DW where available	Peaks in monsoon; bioassay-guided fraction yields THF	Putative HMG-CoA reductase modulation; PPAR-α activation analogs	Krishna 2021 [24]; Dsouza & Nanjaiah 2017 [12]
Phytosterols	β-sitosterol; stigmasterol; campesterol — presence reported	GC-MS; mg/100 g (leaf)	Present across harvests	Compete in micelles; ↓NPC1L1-mediated absorption	Patil 2015 [34]; Ponnamma & Manjunath 2012 [36]— as cited within seasonal study
Pigments	Cyanidin-3-glucoside; β-carotene; lutein	HPLC; mg/100 g; spectral ID	Purple peak in monsoon; higher on Amavasya vs Pournima per lunar study	Antioxidant; ↑ABCA1; ↓LDL oxidation	Patil 2015 [34]; Vasundhara 2018 [54] — as cited within seasonal study
Alkaloids (total)	Total alkaloid content (leaves vs roots) — Justicia spp. comparator	Spectrophotometric; mg CE	Leaves highest; roots lowest among Justicia spp. compared	Anti-inflammatory adjunctive benefits	John*et al.*, 2014 [20] — as cited within seasonal study
Terpenoids/fatty acids	Phytol; neophytadiene; myristic; palmitic; stearic; linoleic; methyl linoleate	GC-MS identification	Reported in active antimicrobial fractions	Membrane disruption; adjunct antimicrobial	Ponnamma & Manjunath 2015 [36]— as cited within seasonal study

Table 3: Mechanistic targets and priority experiments

Constituent/group	Primary target/pathway	Expected lipid effect	Evidence in J. wynaadensis	Evidence analogs	Priority experiments
Phytosterols (β-sitosterol, stigmasterol, campesterol)	Micellar competition; NPC1L1	↓ Intestinal absorption; ↑ fecal sterols	Confirmed presence by GC-MS in leaf extracts	Robust plant sterol literature generalizable mechanism	Caco-2 transport assays; fecal sterol balance in HFD rat; NPC1L1 expression in jejunum
Flavones (THF; luteolin-/apigenin-like)	HMG-CoA reductase; PPAR-α; LDLR	↓ Hepatic synthesis; ↑ HDL; ↑ LDL clearance	THF isolated; antimicrobial and bioactivity suggest tractable dosing	Luteolin/apigenin target data	HepG2 HMG-CoA activity; PPAR reporter assays; hepatic LDLR qPCR/protein after dosing
Anthocyanins (cyanidin-3-glucoside)	ABCA1; antioxidant defense	↑ Reverse cholesterol transport; ↓ LDL oxidation	Seasonal anthocyanin peak documented; purple phenotype during monsoon	Cyanidin literature on ABCA1	THP-1 macrophage efflux to ApoA-I; Ox-LDL inhibition; aortic lesion in ApoE−/− mice
Patent-reported effect (crude extract)	Macrophage Ox-LDL uptake	↓ Foam cell formation	Reduced Ox-LDL uptake in human macrophages (patent)	Anti-atherogenic pathways	Replicate with standardized extract; identify actives by fractionation

Table 4: Safety, tolerability, and interactions roadmap

Domain	Current evidence	Gap	Recommended studies
Culinary safety	Traditional seasonal consumption by Kodava/Kurichiar communities documented ethnobotany in regional accounts	No OECD toxicology	OECD 423/425 acute; 407/408 sub-chronic; genotoxicity; liver/kidney panels
Herb-drug interactions	Overlap with HMG-CoA/NPC1L1 pathways plausible from constituents	No interaction data	*In vitro* CYP/transporters; ezetimibe/statin combination in rodents; monitor CK, ALT/AST
Standardization	Constituents identified; no release specs	Lack of co-markers	Set specs on total phytosterols (GC-MS), THF (HPLC), anthocyanins; stability/ICH

Table 5: Translational study design checklist

Stage	Model/system	Core endpoints	Target engagement
Preclinical efficacy	HFD rat; ApoE−/− mouse; zebrafish lipid model	TC, LDL-C, HDL-C, TG, aortic lesion area, hepatic cholesterol, fecal sterols	NPC1L1 (jejunal), LDLR (hepatic), HMG-CoA activity, ABCA1 (macrophage)
Cellular assays	Caco-2; HepG2; THP-1 macrophages	Cholesterol uptake/synthesis/efflux; Ox-LDL uptake	NPC1L1 transport; SREBP-2/HMG-CoA; PPAR-α reporters; ABCA1 efflux
PK/PD	Rodent oral gavage standardized extract	Plasma/bile levels of THF, sterols, anthocyanins	Tissue distribution; metabolic profiling
Early clinical	Pilot randomized, standardized extract vs placebo	LDL-C primary; HDL-C/TG secondary; Ox-LDL; ApoB/ApoA1; safety labs	Exploratory NPC1L1 surrogates; sterol balance

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