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Review

Pharmacological Properties and Neuroprotective Mechanisms of *Crotalaria verrucosa* L.: an Integrative Approach Bridging Traditional Medicine and Molecular Neuroscience

Kushani Perera[1,2]*, Yugani Rathnayake[1], Thushani Madhurangi[3]

[1]*Department of Life Sciences, NSBM Green University, Homagama, Pitipana, Sri Lanka*
[2]*Department of Chemistry, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka*
[3]*Divisional Hospital, Maligawatte, Colombo 10, Sri Lanka*

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Abstract

Crotalaria verrucosa L. (Fabaceae), or blue rattlepod, is a medicinal plant well-disseminated in tropical and subtropical areas and possesses a wide historical record of traditional use in medicine. Recent phytochemical and pharmacological investigations have revealed an intricate profile of bioactive secondary metabolites, including flavonoids, phenolic acids, terpenoids, phytosterols, and sapogenins, which exhibit impressive antioxidant, anti-inflammatory, hepatoprotective, and cytoprotective properties. This review critically synthesizes current knowledge on the botanical characteristics, phytochemical constituents, and pharmacological activity of *C. verrucosa*, focusing on its potential neuroprotective properties. Key constituents such as gallic acid, caffeic acid, catechin hydrate, vanillic acid, lupeol, and syringic acid act as reactive oxygen species scavengers and inhibitors of lipid peroxidation, thereby countering oxidative stress. Flavonoids, including (-)-epicatechin, vitexin, and isovitexin, are reported to activate the nuclear factor erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) pathway; while β-amyrin, β-sitosterol, and lupeol may influence phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling. Compounds such as caffeic acid, syringic acid, lupeol, and stigmasterol demonstrate anti-inflammatory potential through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways modulation; whereas β-amyrin and β-sitosterol have been implicated in amyloid-β inhibition. Additional evidence suggests a role for phytosterols and sapogenins in cholinergic and GABAergic neurotransmission. Although these mechanistic insights are promising for neuroprotective actions, robust empirical validation in disease-specific *in vivo* and clinical models is still lacking. Therefore, future studies should prioritize bioactivity-guided fractionation, pharmacokinetics, and safety evaluation to advance *C. verrucosa* from traditional medicine toward modern drug discovery for neurodegenerative disorders.

Keywords: Antioxidant; Blue Rattlepod; *Crotalaria verrucosa*; Neuroprotection; Pharmacology; Phytochemistry

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*Corresponding Author: Kushani Perera
Department of Life Sciences, NSBM Green University, Homagama, Pitipana, Sri Lanka
E-mail: kushani.p@nsbm.ac.lk

Introduction

Plants have long served as a prolific source of therapeutic agents, with a significant portion of modern pharmacological drugs tracing their origins to traditional herbal medicines. Among the vast diversity of medicinal floral plants, *Crotalaria verrucosa* L. (Family: Fabaceae), commonly known as blue rattlepod [1], has emerged as a plant of growing scientific interest due to its diverse bioactive compounds and pharmacological potential. It is widely distributed across tropical and subtropical regions of Asia, Africa, and South America [2-4].

Recent phytochemical investigations have disclosed a broad spectrum of secondary metabolites in *C. verrucosa* (CV), including flavonoids, alkaloids, saponins, tannins, and phenolic compounds [5, 6], with a high number of bioactive metabolites having illustrious antioxidant [6], anti-inflammatory [7] and cytoprotective effects [8, 9]. These findings not only define the ethnomedical properties of the selected plant, but also indicate its worth as a potential source of new bioactive molecules for modern therapeutic applications.

Notably, recent evidence suggests that the CV phytochemicals may be behind neuroprotection by antioxidant defense in neuronal tissue, modulation of pro-inflammatory cytokines, and blockade of neurodegenerative pathways. In light of the increased prevalence of neurodegenerative conditions such as Alzheimer's and Parkinson's disease [10-14], there is an urgent need to explore plant extracts that can mitigate neuronal damage and oxidative stress.

While previous reviews have broadly summarized the phytochemistry and pharmacological activities of the *Crotalaria* genus, none has specifically examined CV in the context of neurologic health. Similarly, existing reviews on neuroprotective medicinal plants have focused on widely studied species such as *Bacopa monnieri* (L.) Wettst. [15, 16], *Withania somnifera* [17], and *Curcuma longa* L. [18, 19], leaving a critical gap regarding less explored plants like CV. By systematically correlating its phytochemical profile with mechanistic pathways of neuroprotection, this review offers the first integrative analysis that bridges traditional medicinal use of CV with molecular evidence relevant to neurodegenerative diseases. Accordingly, this review aims to provide an overview of botanical characteristics, phytochemical compositions, and pharmacological activities of CV, with emphasis on its neuroprotective potential. By critically examining its reported mechanisms including antioxidant activity, anti-inflammatory modulation, inhibition of neurotoxic aggregates, and cholinesterase inhibition, this review establishes a foundation for future experimental validation and therapeutic investigation.

Methods

This review employed a narrative synthesis approach, informed by a structured literature search, to gather, analyze, and synthesize existing scientific literature on CV, with an emphasis on its phytochemical composition, pharmacological activities, and potential neuroprotective mechanisms.

Literature search strategy

A comprehensive literature search was carried out using electronic databases including PubMed, Scopus, Google Scholar, and ScienceDirect. Keyword selection was guided by the central themes of the review, including plant taxonomy, phytochemical constituents, and neurological relevance. Core search terms such as "*Crotalaria verrucosa*", "*phytochemicals*", "*neuroprotection*", "*flavonoids*", "*phytosterols*", "*antioxidants*", and "*anti-inflammatory*" were combined using Boolean operators (AND, OR) to broaden and refine the search results. Additional terms related to disease contexts such as "*Alzheimer's disease*", "*Parkinson's disease*", and "*cholinesterase inhibition*" were incorporated to capture studies linking CV to neurological outcomes. Articles were included from 2000 – 2025.

Inclusion and exclusion criteria

Studies were included in this review if they reported either qualitative or quantitative phytochemical profiles of *Crotalaria verrucosa* using validated analytical techniques, evaluated pharmacological activities of CV extracts or isolated compounds through *in vitro*, *in vivo*, or computational models or discussed mechanistic pathways relevant to neuroprotection. Studies were excluded if they focused solely on other *Crotalaria* species or lacked experimental evidence, or if they were duplicate publications or non-peer-reviewed conference abstracts. Data extraction was carried out systematically, with relevant findings organized into three key categories: phytochemical composition (including extraction, identified compounds, and analytical method used), pharmacological activities (detailing extract type, dosage, model systems, and observed outcomes), and mechanistic evidence (associating specific phytochemicals with neuroprotective actions such as ROS scavenging, Nrf2/ARE pathway activation, anti-amyloid effects, and neurotransmitter modulation). All extracted data were critically analyzed to identify consistent trends, knowledge gaps, and emerging mechanistic insights. Visual representations, including schematic diagrams and figures, were developed to summarize the proposed neuroprotective pathways mediated by CV-derived phytoconstituents.

Botanical and ethnomedical overview

CV has been identified as a promising plant species in the genus *Crotalaria* [20]. It is also known as the blue rattlepod, and in the Sri Lankan context, is referred to

as Nilandanahiriya or Yakbairiye [21]. This species is classified within the Kingdom Plantae and falls under the clade of Tracheophytes, Angiosperms, Eudicots, and Rosids. It is further categorized in the Order Fabales, Family Fabaceae, and Sub-family Faboideae, with its placement in the Genus *Crotalaria*. The binomial nomenclature, *Crotalaria verrucosa* L., reflects its taxonomic identity [7,22,23]. It is an herbaceous shrub characterized by simple leaves, dimorphic anthers, and yellow flowers [24].

The plant is native to tropical and subtropical regions of the African and Southern hemispheres. It is found in tropical Burma, Malaya, China [24], and Bangladesh [7]. It is also distributed across parts of India, Punjab, Rajasthan, Gujarat, and in Pakistan, including Sindh, Punjab, Khyber Pakhtunkhwa, and Baluchistan [24]. It has contributed to the Sri Lankan botanical diversity, holding notable ethnobotanical significance [21]. Historically, CV has been widely utilized in traditional medicine across different cultures to manage a broad spectrum of ailments. Leaf juice is traditionally applied for the treatment of scabies and impetigo and is also believed to reduce excessive salivation [25]. In Nigeria, various parts of the plant are used to treat skin infections [8], colic, and flatulence, reflecting its therapeutic role in gastrointestinal and dermatological conditions [25].

Beyond these applications, the plant has been employed in the management of jaundice, cough, biliousness, fever, cardiac ailments, and oral diseases [26]. These ethnomedicinal practices highlight the potential to address inflammatory disorders, infectious diseases, and systemic imbalances with CV.

Phytochemical composition of CV

Supporting the traditional uses of plant CV, phytochemical screening of the different parts of the plant, including leaves, stems, roots, and seeds, has revealed the presence of a diverse array of secondary metabolites [5,6,9,27-29]. The phytochemicals with the screening or analytical technique have been summarized in table1 and table 2.

Qualitative biochemical assays frequently reveal the presence of core classes such as flavonoids, tannins, alkaloids, and saponins across various solvent extracts. Advanced analytical techniques like HPLC-DAD and GC-MS have further characterized specific bioactive molecules, including phenolic acids (e.g., gallic acid, vanillic acid, caffeic acid), flavonoids (e.g., catechins, quercetin, kaempferol), and phytosterols (e.g., β-sitosterol, campesterol, stigmasterol). These compounds are of particular interest due to their well-documented pharmacological properties. Despite the recognized pharmacological potential of CV, the utilization of advanced analytical methods for the precise identification and characterization of its phytochemical constituents remains inadequately explored.

Reported pharmacological activities of CV plant

CV has demonstrated a broad spectrum of pharmacological effects, supported by both *in vitro* and *in vivo* studies. These effects are primarily attributed to its rich composition of flavonoids, phenolic acids, alkaloids, and terpenoids and are summarized in table 2.

Abbreviations: GAE - gallic acid equivalent, TPC - total phenolic content, AAE - ascorbic acid equivalents, TFC - Total flavonoid content, QE- quercetin equivalent, RE-rutin equivalents, ZOI - Zone of inhibition

Potential neuroprotective effects and possible mechanisms

Neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and Hunting-

Table 1. Summary of the phytochemical constituents identified in the phytochemical screening from different extracts of CV

Year	Leaf extract	Phytochemicals/ Classes identified										Ref.
		Alkaloids	Phenols	Saponins	Steroids	Flavonoids	Tannins	Terpenes	Reducing sugars /Carbohydrates	Proteins	Resins	
2013	Ethanol	+	-	+	-	+	+	-	+	+	+	[9]
2015	Methanol (95%)	+	+	+	+	+	+	-	+	-	-	[28]
2015	n-butanol	+	+	-	+	+	+	+	+	+	-	[5]
2019	Ethanol	+	+	-	-	+	+	+	-	-	-	[27]
	Acetone	+	+	-	+	+	-	-	+	-	-	[29]
	Chloroform	+	-	-	+	+	-	-	+	-	-	[29]
	Ethanol	+	+	+	+	+	+	-	+	-	-	[24]

Table 2. Summary of the phytochemical constituents identified in the quantitative analysis

Year	Leaf extract	Phytochemicals/ Classes identified	Method	Ref.
2016	Methanol	Polyphenolic compounds Phenolic acids Gallic acid, Vanillic acid, Caffeic acid, Syringic acid Flavonoids (+)-Catechin hydrate, (-)-Epicatechin Other Vanillin	HPLC-DAD analysis (High-Performance Liquid Chromatography with Diode Array Detection)	[6]
2021	Ethyl acetate extract	Fatty alcohols and Fatty acids 3,7,11-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 3-Chloropropionic acid, tridec-2-ynyl ester, i-Propyl 9,12,15-octadecatrienoate, 2-((Octan-2-yloxy) carbonyl) benzoic acid Hydrocarbons Squalene Phytosterols Campesterol, stigmasterol, β - sitosterol Saponin (25R)-5. Alpha. Spirostan2.alpha.,3. beta-diol (SSD)	GC-MS	[26]

Table 3. Summary of reported pharmacological effects of the CV plant

Tested effect	Leaf extract	Observed results	Conclusion	Ref.
Antioxidant activity	Methanol	400 µg/mL of methanolic extract showed: • Total antioxidant capacity - 19.58 mg/g • TPC = 152.18 mg/g GAE • TFC = 184.51 mg/g QE DPPH free radical scavenging assay • IC_{50} = 533.74 µg/mL	Indicates moderate antioxidant capacity with flavonoid content exceeding total phenolic content	[6]
	Zinc oxide nanoparticles (ZnONps) derived from ethanolic extract	• TPC = 41 ± 1.1 GAE/g • TFC = 8 ± 0.2 RE/g	The ethanolic leaf extract-derived ZnONPs are rich in phenolic compounds and flavonoids	[30]
Antibacterial effect	n-butanol	At 100 µg/mL of plant extract, ZOI: • *Bacillus subtilis* and *Klebsiella pneumonia* = 15 mm • *Escherichia coli* = 13 mm • *Proteus vulgaris* = 14 mm • *Pseudomonas aeruginosa* = 12 mm • Gentamycin has been used as the standard.	Suggests broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria compared with gentamycin	[5]
	Methanol	Three bacterial strains have been tested at 250 and 350 µg/disc: *Bacillus cereus, Streptococcus pneumoniae, and Shigella dysenteriae* At 350 µg/disc, a 17 mm ZOI has been observed against *Bacillus cereus*. Kanamycin (standard) = 31 mm ZOI.	It indicates potential antibacterial activity, particularly against *B. cereus*.	[8]

Antibacterial effect	ZnONps ethanolic extract	Highest ZOI - observed at 100 µg/mL. Anti-bacterial effects have been exhibited against *P. valgaris*, *E. coli*, *K. pneumoniae,* and *S. aureus,* respectively, showed ZOI 17 ± 1.1, 15 ± 0.9, 17 ± 0.5, and 18 ± 0.3 mm. Streptomycin had been used as a standard. ZOI for standard, respectively, 18 ± 1.4, 16 ± 1.7, 20 ± 1.2, and 21 ± 0.9 mm. Scanning electron microscopy (SEM) revealed morphological disruption of bacterial cells following nanoparticle treatment.	ZnONps enhanced the antibacterial efficacy of ethanolic extract by increasing surface interaction with bacterial cells.	[30]
Hypoglycemic effect	Ethanol	In vitro Glucose absorption varied dose-dependently. In vivo: At 500 mg/kg, $BaSO_4$ milk travelled farther in the gut (enhanced motility). The sucrose absorption assay showed reduced unabsorbed sucrose and lower glucose levels from residual sucrose.	Extract improved intestinal motility and carbohydrate digestion, reducing sucrose malabsorption.	[27]
	Ethanol	Oral administration of CV extract (100, 250, and 500 mg/kg) for 21 days in alloxan-induced diabetic Wistar rats produced a dose-dependent reduction in blood glucose levels. In comparison, the standard drug glibenclamide (2.5 mg/kg) significantly ($P < 0.001$) lowered blood glucose as early as day 7, demonstrating markedly greater potency at a much lower dose	The hypoglycemic effect of CV extract was significant but considerably weaker in potency compared to glibenclamide. Whereas the standard drug achieved glucose reduction at 2.5 mg/kg, CV extract required a 200-fold higher dose (500 mg/kg) to show comparable effects. This indicates that while CV contains active hypoglycemic constituents, its efficacy remains modest relative to established antidiabetic agents.	[31]
Anticoagulant properties	Methanol	An *in vitro* thrombolytic assay was conducted to evaluate the clot lysis potential of crude extracts against the positive control streptokinase. The thrombolytic activity of the positive control and plant extract showed 79.55 ± 9.09% and 35.88 ± 8.94 % clot lysis, respectively.	*Crotalaria verrucosa* exhibits moderate clot-dissolving potential, supporting its traditional medicinal use and warranting further investigation as a natural thrombolytic agent.	[28]
Antiproliferative effect	Ethanol	MTT Assay. Demonstrated cytotoxic activity against HeLa and DU145 cancer cell lines with IC_{50} values of 22.30 µg/mL and 15.72 µg/mL, respectively. Green-synthesized ZnONp derived from the same extract exhibited enhanced cytotoxicity, achieving maximum growth inhibition at 100 µg/mL, with significantly lower IC_{50} values of 7.07 µg/mL for HeLa and 6.30 µg/mL for DU145 cell lines.	Suggests that nanoparticle formulation can potentiate the anticancer efficacy of plant-based extracts, possibly by improving cellular uptake and increasing the reactive surface area.	[30]
	Methanol	Dose-dependent cell proliferation was observed in the MTT assay. The methanolic extract of CV exhibited significant cytotoxic effects, with the highest inhibition of cell growth (93.2%) observed at a concentration of 2.5 mg/mL. The IC_{50} value = 0.83 mg/mL.	Indicates a moderate cytotoxic effect, suggesting the potential of CV as a source of bioactive compounds with anticancer properties.	[8]

Sedative effect	Ethanol	Brewer's yeast-induced pyretic model: Swiss albino rats treated at 250 and 500 mg/mL concentrations exhibited moderate protective effects against hyperthermia.	At 250 and 500 mg/mL concentrations, it exhibited moderate protective effects against hyperthermia.	[31]
	Ethanol	The sedative activity of the extract was evident from the observed reduction in locomotor activity across different doses (100, 250 & 500 mg/mL). This effect was consistently demonstrated in the hole cross-test and the open-field tests.	This indicates a dose-dependent suppression of spontaneous motor activity, characteristic of sedative properties	[31]
Hepatoprotective effect	Ethanol	Paracetamol-induced hepatotoxic rats treated with ethanolic extract at 100, 200, and 400 mg/kg lowered serum enzyme levels (AST, ALT, and bilirubin) and increased liver enzymes, superoxide dismutase, glutathione, and glycogen levels. Histopathological findings have demonstrated improved liver architecture, reduced necrosis, and degeneration compared to the paracetamol-induced group.	Ethanol extract at the tested concentrations led to the reduction of oxidative stress and restoration of liver function	[9]
Anti-inflammatory and wound healing properties	Aqueous	Xylene-induced ear oedema: 54% reduction at 600 mg/kg. Carrageenan-induced paw oedema: similar effects. In vitro: 70% (heat-induced hemolysis), 69% (protein denaturation) at 500 µg/mL.	Possesses both membrane-stabilizing and protein-stabilizing properties, supporting its traditional use as an anti-inflammatory agent	**[7]**
	Aqueous	Wistar rats Incision wound model – Control group breaking strength (278.95 g) has been increased to 359.20 g and 388.35 g, respectively, at the 400 mg/kg and 800 mg/kg of CV extract. Dead space wound model- increased granulation tissue breaking strength and hydroxyproline content, total protein and dry weight, with more pronounced improvements at 800 mg/kg dose. Catalase and glutathione synthase activity have significantly increased. Excision wound model - Enhanced the wound contraction at 800 mg/kg up to the 16th day with a 4-day interval and reduced the epithelialization period.	Exhibits wound healing activity through enhancement of tensile strength, granulation tissue formation, collagen synthesis, and antioxidant enzyme levels. It also effectively counteracts the wound healing inhibition caused by dexamethasone, indicating its strong anti-inflammatory and regenerative potential in wound models.	[25]

ton's disease are characterized by progressive loss of neuronal structure and function, often leading to cognitive, motor, and behavioral impairments [14,32,33]. The underlying mechanisms are complex and multifactorial, involving oxidative stress, neuroinflammation [34, 35], mitochondrial dysfunction, protein misfolding [36,37], excitotoxicity, and apoptosis [38].

Despite extensive research, current pharmacological interventions remain largely palliative, providing symptom relief without halting or reversing neuronal degeneration.

Given this multifactorial pathology, phytochemicals with antioxidant, anti-inflammatory, anti-amyloid, and neurotransmitter-modulating properties

are promising therapeutic candidates. Several such compounds have been identified in CV, providing a rationale for investigating its neuroprotective potential. Although direct empirical validation in CV is still lacking, the presence of these bioactive molecules allows hypothesis-driven mapping of potential mechanisms.

Antioxidant effect in neuroprotection

The central nervous system is more susceptible to oxidative stress due to limited repair mechanisms, low levels of endogenous antioxidants, inability of neuronal cells to undergo division, and a high membrane surface-to-volume ratio [39]. Neuroprotective strategies commonly focus on mitigating oxidative stress and excitotoxicity, both of which contribute significantly to neuronal death and accelerate the progression of neurodegeneration. Consequently, the use of glutamate receptor antagonists and antioxidant agents has shown considerable promise in both experimental research and clinical therapy [39]. CV is rich in non-enzymatic antioxidants; gallic acid, caffeic acid, catechin hydrate, vanillic acid, lupeol, and syringic acid, and enzyme-mediated antioxidants such as (-) epicatechin, vitexin, and isovitexin [26].

Compounds such as gallic acid, caffeic acid, catechin hydrate, vanillic acid, and syringic acid act as direct ROS scavengers, neutralizing free radicals before they inflict cellular damage[40-42]. These polyphenolic compounds possess hydroxyl groups capable of donating hydrogen atoms to free radicals, thereby stabilizing them and terminating radical chain reactions [41-44]. Such an antioxidant-rich composition suggests potential neuroprotective value by mitigating oxidative injury and thereby may slow the progression of neurodegenerative diseases.

Studies have demonstrated that inhibition of the Nrf2/ ARE (nuclear factor erythroid 2-related factor 2/ antioxidant response element) pathway induces oxidative stress, which subsequently contributes to the pathogenesis of neurodegenerative diseases, including Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease [45-48]. In addition to direct scavenging activity, flavonoids such as (–) -epicatechin, vitexin, and isovitexin have been shown in other plant systems to contribute to antioxidant defense through enzyme modulation mechanisms. These compounds have been shown to activate the Nrf2/ARE signaling pathway, which is a regulator of antioxidant gene expression [45,46,49]. However, no direct experimental evidence currently confirms the activation of Nrf2/ARE by CV extracts. Therefore, this mechanism should be interpreted as a speculative hypothesis based on the reported effects of structurally similar phytochemicals. Activation of Nrf2 leads to the upregulation of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), further enhancing the cellular ability to detoxify ROS and maintain redox balance [50].

Inhibition of macromolecular damage by

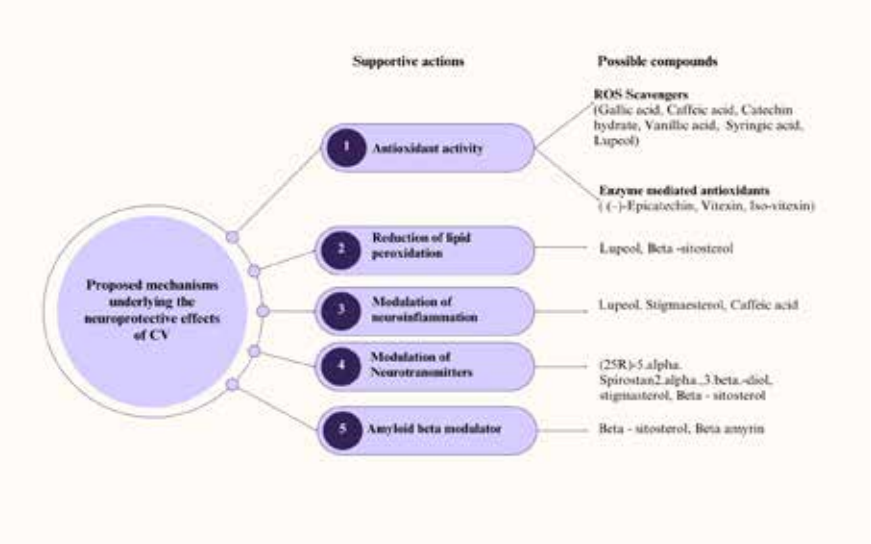

Figure 1. Proposed mechanisms underlying the neuroprotective effects of CV. The neuroprotection can be approached by multiple pathways, including (1) antioxidant activity through ROS scavenging (Gallic acid, Caffeic acid, Catechin hydrate, Vanillic acid, *Syringic acid*) and enzyme modulation ((–)-Epicatechin, Vitexin, Isovitexin); (2) reduction of lipid peroxidation (Lupeol, β-sitosterol); (3) modulation of neuroinflammation (Lupeol, Stigmasterol, Caffeic acid); (4) modulation of neurotransmitter systems ((25R)-5α-spirostan-2α,3β-diol); and (5) inhibition of amyloid beta aggregation (β-sitosterol, β-amyrin). (Author compilation)

phytochemicals in neuroprotection

The brain is an organ with a high lipid content, making it particularly vulnerable to oxidative damage via lipid peroxidation [51]. Among various macromolecular damages associated with neurodegeneration, the inhibition of lipid peroxidation has been extensively investigated as a critical therapeutic target in neurodegenerative diseases [52-54]. In this context, two bioactive compounds found in CV*:* lupeol and β-sitosterol, have emerged as potential lipid peroxidation inhibitors. An *in vitro* study conducted by Shi et al. (2013) demonstrated that β-sitosterol, when incorporated into cell membranes, enhances resistance to oxidative stress and inhibits lipid peroxidation through estrogen receptor-mediated phosphatidyl inositol 3-kinase (PI3K)/glycogen synthase kinase 3 (GSK3β) signaling. This signaling cascade plays a key role in neuronal survival by regulating oxidative stress responses and promoting anti-apoptotic mechanisms.

Similarly, lupeol, a pentacyclic triterpenoid present in CV, has shown significant antioxidant potential [51,55]. Studies using lupeol extracted from *Crateva adansonii* and *Ficus pseudopalma* revealed that it exhibits superior antioxidant capacity to standard ascorbic acid. Lupeol has effectively reduced lipid peroxidation, DNA damage, and protein oxidation; while simultaneously increasing levels of reduced glutathione (GSH), a key cellular antioxidant [56,57]. Together, these findings suggest that lupeol and β-sitosterol are key phytochemicals that may contribute to the inhibition of lipid peroxidation, thereby offering potential neuroprotective benefits. However, these effects have not yet been experimentally validated in CV and their specific role in mitigating neuronal damage remains to be established.

Modulation of neuroinflammation by phytochemicals

The NF-κB pathway is recognized as one of the key molecular mechanisms involved in neuroinflammation. In Alzheimer's disease and Parkinson's disease, the aggregation of amyloid proteins and α-synuclein, respectively, induces the activation of microglia into the pro-inflammatory M1 phenotype. This activated state leads to the release of cytokines such as TNF-α, IL-1β, and IL-6, contributing to neuronal damage through inflammatory processes [58, 59]. Therefore, inhibiting the NF-κB pathway is considered critical for protecting neuronal cells from inflammation-induced degeneration. Several *in vivo* and *in vitro* studies [60, 61] have shown that lupeol has a strong capacity to reduce these pro-inflammatory cytokines and exhibits promising therapeutic potential for anxiety and depression. Given these findings, investigating the neuroprotective effects of lupeol in the context of neurodegenerative disorders is both relevant and warranted. CV leaves are rich in caffeic acid. Caffeic acid, isolated from *E. annuus* leaf, has demonstrated a significant protection of PC12 neuronal cells from H_2O_2-induced oxidative stress and membrane damage, with the highest protection observed at 40 µg/mL [62]. The derivatives of caffeic acid have shown more effective penetration of the blood-brain barrier [63]. Stigmasterol, a triterpene, is another potent anti-inflammatory compound found in several medicinal plants [64, 65]. The leaf extract of *Momordica charantia* is rich in stigmasterol, and it has reduced the ROS production and downregulated the expression of MAPK-related proteins, which leads to decreased cell apoptosis and restoration of normal cell cycle progression [64]. Dysregulation of neurotransmitters, including dopamine, serotonin, glutamate, γ-aminobutyric acid (GABA), and acetylcholine (ACh), is a well-established hallmark in the pathogenesis of various neurological and psychiatric disorders [66, 67]. Although early findings have proven that low serotonin, norepinephrine, and dopamine levels are associated with depression, recent research highlights the role of glutamatergic hyperreactivity and reduced GABAergic function in the cortex of depression patients [68-71]. A GABAergic deficit, associated with decreased expression of the enzyme glutamic acid decarboxylase, has been identified in postmortem brains of patients with schizophrenia [72]. Findings proved that loss of cortical acetylcholine level is associated with the degeneration of cholinergic neurons in Alzheimer's disease patients [73]. The primary excitatory and inhibitory neurotransmitters, glutamate and GABA, are directly associated with epilepsy [74]. Across these neurological disorders, a consistent finding is that enhancing GABAergic neurotransmission may serve as a promising therapeutic strategy.

Although CV contains sterols and sapogenins known elsewhere to modulate cholinergic, GABAergic, and serotonergic systems, no studies have yet characterized these effects in CV-derived preparations. The absence of electrophysiological, neurochemical, or behavioral data prevents definitive attribution of neurotransmitter-level modulation to this species. Systematic evaluation of CV extracts and purified compounds in neuronal and receptor-specific assays is therefore required.

Modulation of neurotransmitters

Surprisingly, the presence of phytosterols stigmasterol, β-sitosterol, and steroidal sapogenin; Saikosaponin d (SSD), suggests potential modulation of neurotransmitter systems relevant to neurological disorders. Stigmasterol, purified from the microalga *Phormidium retzii*, has demonstrated significant acetylcholinesterase inhibitory activity, exhibiting 81.2 ± 0.08% inhibition with an IC_{50} value of 0.214 µM in an *in*

vitro assay. These findings suggest its potential as a safer therapeutic candidate for Alzheimer's disease with minimal side effects [75]. A preclinical study conducted using mice has reported that stigmasterol demonstrated the beneficial effects in the context of Alzheimer's disease by the enhancement of the cholinergic neurotransmission system via the activation of estrogen or N-methyl-D-aspartate (NMDA) receptors [76].

Moreover, stigmasterol extracted from *Artemisia indica* Linn was investigated for its modulatory effects on various GABA-A receptor subtypes (α2β2γ2L, α4β3δ, and α4β3) using *Xenopus laevis* oocyte expression systems, behavioral assays in mice, and computational modeling [65]. *In vitro* studies demonstrated that stigmasterol significantly enhanced GABA-induced currents, particularly in the extrasynaptic α4β3δ subtype, suggesting a key role of the δ subunit in its efficacy. *In silico* docking confirmed stigmasterol's binding to known modulator sites, notably in the transmembrane region. *In vivo*, stigmasterol (0.5–3.0 mg/kg, intraperitoneally) exhibited pronounced anxiolytic and anticonvulsant effects comparable to allopregnanolone [65]. The antidepressant effects of β-sitosterol derivatives have been reported at a dose of 4 mg/kg in adult male mice, with the underlying mechanism involving modulation of the serotonergic, dopaminergic, and GABAergic neurotransmitter systems [77].

SSD is also known as gitogenenin [78], which belongs to the class of steroidal sapogenins. The neuroprotective activity of sapogenin has been studied over the last two decades [79]. Although the direct evidence is limited to evaluating its effects, analogues of spirostan compounds suggest that it can modulate neurotransmitter systems affecting cognition. Sarsasapogenin, a structurally related sapogenin, has improved learning in memory-deficient rats by increasing muscarinic acetylcholine receptor density in the cortex and hippocampus [80]. Furthermore, sarsasapogenin, identified in the aqueous extract of *Asparagus racemosus*, has been reported to exert concentration-dependent inhibitory effects on the key enzymes, acetylcholinesterase and butyrylcholinesterase, both of which are critically involved in the pathogenesis of Alzheimer's disease [81]. The 5-ene analogue of the SSD, which is also called diosgenin, has demonstrated the ability to ameliorate behavioral impairments in models of stress and psychosis by restoring dopamine and serotonin homeostasis and suppressing excessive glutamate release, while also protecting against microglia-mediated dopaminergic neuron loss in an inflammatory model of Parkinson's disease [82]. Despite the structural and pharmacological relevance of SSD concerning similar bioactive compounds, a significant empirical gap remains regarding its neurotherapeutic potential. Accordingly, rigorous experimental studies are warranted to investigate the effects of purified SSD fractions, particularly in the context of Alzheimer's disease and other neurological disorders.

Collectively, these cross-species findings highlight plausible mechanisms through which stigmasterol, β-sitosterol, and gitogenin may exert neuroprotective effects; however, their roles within CV remain speculative and unverified experimentally. Dedicated studies using purified fractions from CV are therefore warranted to confirm these hypothesized effects, particularly in the context of Alzheimer's and other neurodegenerative disorders.

Anti-beta amyloidogenic effect

Alzheimer's disease is characterized by the pathological accumulation of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, which subsequently lead to the disruption of synapses, neuroinflammation, and neuronal death [33]. The two main secondary metabolites, including amyrin and β-sitosterol, are extracted from the ethyl acetate extract of the CV, which has an anti-amyloid effect [26].

Pentacyclic triterpenoid, β-amyrin, was shown to prevent amyloid-β (Aβ)-induced synaptic dysfunction. Specifically, β-amyrin blocked Aβ-induced long-term potentiation impairment in mouse hippocampal slices in a study conducted in 2019 [83]. It was suggested that the protective effect is associated with the restoration of the inhibited intracellular PI3K/Akt signaling pathway and inhibition of the upregulated glycogen synthase kinase-3β (GSK-3β) affected by the amyloids [83]. β-Amyrin has been shown to act as a cannabinoid receptor agonist, notably (CB_1/CB_2) receptors, which modulates microglial activity [84]. A recent study of *Passiflora edulis* extract (passion fruit), which contains α-amyrin, found that the extract protected neurons against Aβ toxicity [85]. These findings collectively suggest that amyrins may reduce Aβ burden with β-amyrin primarily attenuating synaptotoxicity, and α-amyrin enhancing Aβ clearance mechanisms. However, the absence of clinical studies evaluating amyrins in Alzheimer's disease patients highlights a significant gap in translating these preclinical findings to human models. The presence of β-amyrin in the leaves of CV, opens a promising avenue for future research to explore the potential of CV-derived amyrins in modulating β-amyloid pathology associated with Alzheimer's disease.

A study by Ye et al. (2020) demonstrated that four weeks of β-sitosterol treatment in APP/PS1 double-transgenic mice significantly reduced amyloid plaque burden and led to notable improvements in spatial learning and memory performance. These findings are supported by evidence linking its modulation of cholesterol metabolism to reduced Aβ for-

mation. By altering lipid raft composition, β-sitosterol is known to inhibit β-secretase activity and promote non-amyloidogenic processing of amyloid precursor protein [86].

Furthermore, β-sitosterol isolated from *Polygonum hydropiper* has also been demonstrated to have anti-amyloidogenic properties *in vitro*, *in vivo* and *in silico* analysis [89]. Theoretically, the presence of β-sitosterol in CV may confer similar neuroprotective effects, addressing the requirement of more advanced research in that area.

Limitations and future directions

A limited number of studies were found in which phytochemical identification was performed using advanced techniques rather than the screening of phytochemicals. Despite the promising phytochemical profile of CV, several limitations must be acknowledged when considering its potential as a neuroprotective agent.

While compounds such as β-amyrin, β-sitosterol, and flavonoids identified in CV are individually known to exhibit antioxidant, anti-inflammatory, and neuroprotective properties, there is currently a lack of direct experimental evidence assessing the plant's efficacy in validated *in vitro* or *in vivo* models of neurodegenerative diseases, such as Alzheimer's or Parkinson's disease. The presence of pyrrolizidine alkaloids in the seeds of CV plant [90] raises additional safety concerns, and although leaves of CV are considered to have lower toxicity, comprehensive toxicological assessments are still required to ensure their safe therapeutic application. Pyrrolizidine alkaloids are a well-characterized group of hepatotoxic and genotoxic constituents commonly found in many *Crotalaria* species. The hepatic bioactivation of cytochrome P-450 enzymes provides reactive dehydropyrrolizidine metabolites which have the ability to covalently react with cellular macromolecules to precipitate hepatic sinusoidal obstruction syndrome, hepatocellular necrosis, and DNA adduct formation. Chronic exposure has been correlated with the carcinogenic and mutagenic effects in test models. The alkaloid profile and concentration in CV are subject to variation based on part of the plant, plant growth conditions, and extraction; however, even at trace concentrations, it should be used with caution when evaluating medicinal or nutraceutical use. It follows that quantification, detoxification measures, and toxicokinetic investigations are indispensable before considering CV-based preparations safe as therapeutic or dietary agents [91,92]. Moreover, the variability in phytochemical composition due to differences in geographical origin, plant maturity, and extraction methods complicates standardization, which is critical for developing reproducible and clinically relevant formulations. Another major limitation lies in the limited

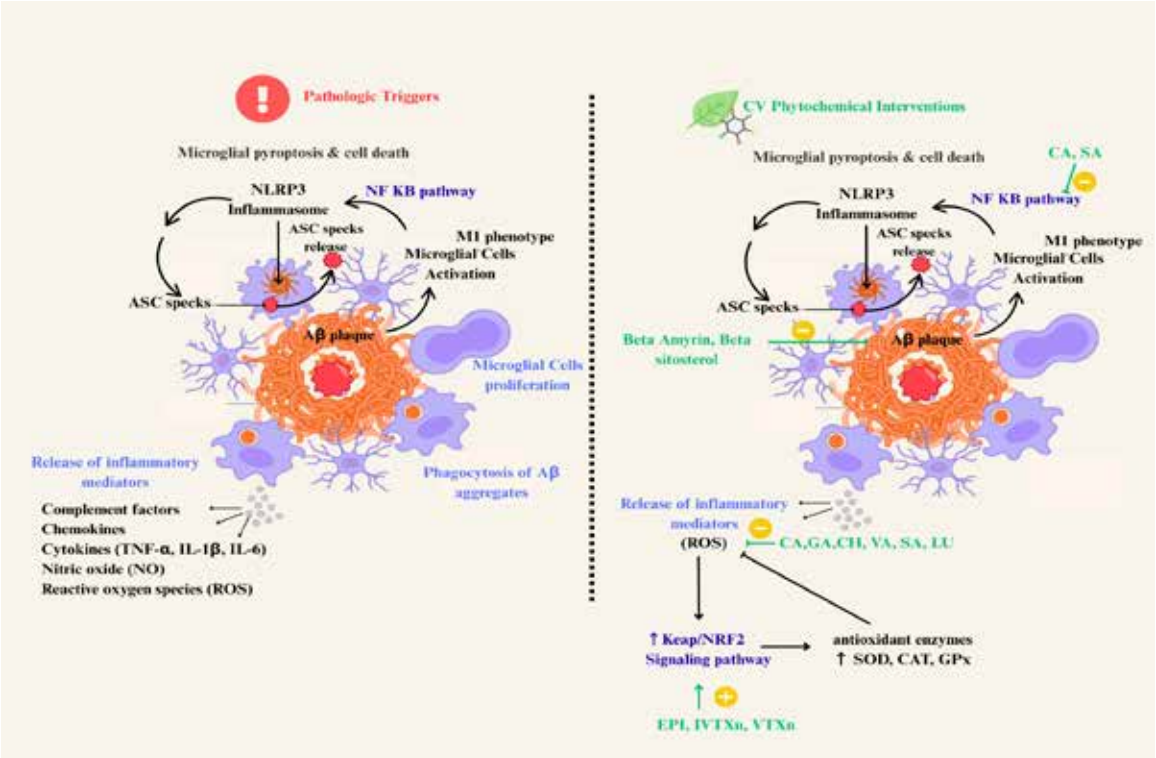

Figure 2. Possible mechanistic pathways of neuroprotection by phytoconstituents from CV. This schematic diagram illustrates the interconnected molecular mechanisms involved in neuronal inflammation and cell death, with a focus on the potential neuroprotective actions of phytochemicals from CV. Key pathological contributors, including oxidative stress, microglial activation, and amyloid-β plaque formation, converge to activate the NF-κB and MAPK pathways, which further promote neuroinflammation (via NLRP3 inflammasome and cytokines like TNF-α, IL-1β, and IL-6) and neuronal apoptosis. *CA: Caffeic acid, GA: Gallic acid, CH: Catechin hydrate, VA: Vanillic acid, SA: Syringic acid, LU: Lupeol, EPI: Epicatechin, IVTXn: Isovitexin, VTXn: Vitexin, SSD: ((25R)-5.alpha. Spirostan2.alpha.,3.beta.-diol* [87,88].

mechanistic insight into how CV-derived compounds interact with key molecular pathways involved in neurodegeneration, even though CV plant shares similar compounds that possess neuroprotective activity, such as PI3K/Akt, GSK-3β, MAPK, and Nrf2 signaling. Moreover, the absence of clinical studies means that the therapeutic relevance of CV in human neurodegenerative conditions remains speculative.

From a translational perspective, additional barriers must also be considered. Many of the identified phytochemicals, including flavonoids and triterpenoids, often suffer from low oral bioavailability, rapid metabolism, and poor aqueous solubility, which may limit their systemic exposure and therapeutic efficacy [93-95]. Furthermore, blood brain barrier penetration remains a major challenge; only a few of these compounds have demonstrated the capacity to reach effective concentrations in the central nervous system [94,95].

Addressing these empirical gaps through systematic pharmacological, toxicological, and clinical investigations will be essential to validate the neuroprotective potential of this underexplored medicinal plant.

Conclusion

The CV plant is rich in phytochemicals encompassing flavonoids, phenolic acids, phytosterols, triterpenoids, and sapogenins that collectively support diverse pharmacological activities. Although most evidence arises from non-neuronal models indicating antioxidants, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and anticancer effects, these emerging findings offer constituents such as β-sitosterol, β-amyrin, lupeol, stigmasterol, and SSD from the plant. These constituents are known to modulate oxidative stress, lipid peroxidation, neuroinflammation, amyloid aggregation, and cholinergic and GABAergic neurotransmission in other plant species, offering a rationale for exploring similar effects in CV. Moving forward,

While pharmacological studies of CV provide a compelling rationale for its therapeutic exploration, particularly in Alzheimer's disease and related disorders, the absence of disease-specific experimental and clinical validation presents a major knowledge gap. Furthermore, the potential presence of toxic alkaloids, variability in phytochemical content, and lack of standardized extracts highlight the need for cautious and evidence-based development. To fully harness its neuroprotective potential, future research should prioritize bioactivity-guided fractionation of neuroactive fractions, mechanistic studies in neuronal cell lines and animal models of neurodegenerative diseases, toxicological and pharmacokinetic profiling to ensure safety and bioavailability, and, ultimately, clinical translation. By addressing these gaps, *C. verrucosa* could progress from an ethnomedicinally-valued plant to a scientifically-validated source of neuroprotective leads, advancing the development of plant-based therapeutics for neurodegenerative disorders.

Conflict of Interests

No conflicts of interest are associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Using Artificial Intelligence (AI)

The authors used artificial intelligence (AI)–assisted tools solely to improve grammar, spelling, and language clarity. No AI system was employed for data analysis, interpretation, or content generation. The authors take full responsibility for the scientific accuracy and integrity of the manuscript.

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