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In Silico Screening of *Ruellia tuberosa* Phytochemicals as Neuroimmune-Modulating Drug Candidates for Multiple Sclerosis Therapy

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease characterized by neuroinflammation, demyelination, and progressive neurological decline. Current pharmacotherapies offer limited efficacy and often induce adverse effects, highlighting the need for safer, multi-target alternatives. This study employed an *in silico* approach to evaluate phytochemical compounds from *Ruellia tuberosa* as potential neuroimmune-modulating drug candidates for MS therapy. Five bioactive compounds, which are hentriacontane, nonacosane, campesterol, β-sitosterol, and stigmasterol, were identified through the KNApSAcK database and assessed for drug-likeness via Lipinski's Rule of Five. All fulfilled key criteria, though high LogP values were noted. ProTox-II analysis indicated acceptable toxicity profiles for campesterol, β-sitosterol, and stigmasterol, warranting further exploration. SwissTargetPrediction and PASS Online analyses revealed that these compounds interact predominantly with nuclear receptors, cytochrome P450 enzymes, and oxidoreductases, pathways strongly implicated in MS pathophysiology. The predicted pharmacological activities included immunosuppression, caspase-3 stimulation, prostaglandin-E2 9-reductase inhibition, and HMOX1 expression enhancement. These mechanisms are relevant to modulating neuroinflammation, oxidative stress, and steroid hormone regulation in MS. Collectively, these findings highlight the potential of *Ruellia tuberosa* phytochemicals as orally active, multi-target agents that may serve as complementary therapies in MS management. Further in vitro and in vivo validation is recommended to substantiate their efficacy and pharmacokinetic properties.

Keywords: *Ruellia tuberosa*, multiple sclerosis, in silico screening, phytochemicals, natural drug candidate

INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system (CNS), widely recognized as the leading non-traumatic cause of disability in young adults. The disease is marked by aberrant immune activation against myelin components, leading to progressive neuroinflammation, axonal injury, and functional neurological decline (1). Although the precise etiology of MS remains unknown, current evidence supports a multifactorial origin involving both genetic susceptibility and environmental exposures. Factors such as infections, vitamin D deficiency, and tobacco

use are known to disrupt immune tolerance and trigger autoreactive immune responses that contribute to demyelination and neuronal degeneration (2) A key element in MS pathogenesis is the chronic inflammatory microenvironment within demyelinating lesions, which facilitates the release of pro-inflammatory cytokines and the overproduction of reactive oxygen and nitrogen species (ROS and RNS). These mediators not only exacerbate inflammation but also induce oxidative stress, creating a self-amplifying loop that promotes neuronal cell death and demyelination. The interplay between oxidative stress and inflammation is increasingly recognized as a central mechanism driving both disease onset and progression in MS (3)

Although multiple sclerosis remains an incurable disease, the development of novel therapeutic strategies to mitigate its symptoms and delay disease progression remains a critical area of ongoing research. Current treatment strategies for multiple sclerosis primarily focus on managing acute relapses, employing disease-modifying therapies (DMTs) to reduce disease activity and progression, and addressing symptomatic issues, through a combination of pharmacological and non-pharmacological interventions (4) The pharmacological approaches of multiple sclerosis treatment is focused on the use of disease-modifying therapies, including immunomodulatory and immunosuppressive agents such as interferon-β, dimethyl fumarate, natalizumab, and fingolimod. These agents function by modulating immune activity to reduce relapse frequency, delay the progression of neurodegeneration, and mitigate inflammatory demyelinating episodes. Recent advances have led to the development of targeted monoclonal antibodies, such as ocrelizumab, which selectively deplete CD20-positive B cells, thereby offering therapeutic benefit across both relapsing-remitting and primary progressive MS phenotypes. However, the clinical efficacy of these agents is influenced by interindividual variability, treatment adherence, and the need for ongoing pharmacovigilance due to the risk of immune-related adverse effects (5).

Given the therapeutic limitations, suboptimal efficacy in progressive forms, and adverse effect profiles of current disease-modifying therapies, there is an urgent need to identify novel bioactive compounds with improved safety and efficacy. Natural products, particularly those derived from plants, have garnered increasing interest due to their multifaceted pharmacological activities, including immunomodulatory, anti-inflammatory, and neuroprotective effects. Experimental and preclinical studies have demonstrated that several phytochemicals can modulate key molecular targets involved in MS pathogenesis, supporting their potential as scaffolds for the development of next-generation therapeutics (6)

Phytochemicals derived from plants phytochemical compounds offer considerable therapeutic potential in the treatment of multiple sclerosis, primarily to their anti-inflammatory, immunomodulatory, and neuroregenerative properties. Evidence from both preclinical and clinical investigations suggests that these bioactive compounds can modulate dysregulated immune pathways, attenuate neuroinflammation, and facilitate remyelination. By targeting multiple mechanisms involved in disease progression, plant-derived compounds represent promising adjunctive candidates that may complement existing treatment regimens and mitigate the adverse effects often associated with long-term use of conventional pharmacotherapies (7) The therapeutic benefits of plant-derived compounds, which include reducing inflammation and oxidative stress as well as improving neurological function, emphasize the need for further investigation and clinical evaluation to confirm their efficacy and safety in the treatment of multiple sclerosis (8)

Ruellia tuberosa L. is a medicinal plant commonly used in traditional medicine across tropical regions, including Indonesia. It is traditionally applied to treat conditions

such as diabetes, hypertension, and inflammation. Research has shown that hydroethanolic root extracts of *R. tuberosa* can improve kidney histology and reduce oxidative stress markers like malondialdehyde (MDA) and TNF-alpha in diabetic animal models. These effects are linked to the plant's content of triterpenoids, phytosterols, and flavonoids, which possess antioxidant and anti-inflammatory properties. This suggests its potential for broader therapeutic applications beyond traditional use. Exploring drug candidates from *Ruellia tuberosa* offers several advantages, including the potential for safer and more cost-effective therapies with fewer adverse effects than synthetic drugs. Compounds from this plant have shown the ability to modulate immune responses and reduce oxidative damage, both of which are important in treating chronic diseases such as diabetes and possibly neurodegenerative disorders. Its long-standing traditional use, supported by early scientific evidence, provides a solid basis for further molecular and clinical research to identify active constituents and clarify their mechanisms, supporting the development of new phytopharmaceuticals (9). Phytochemical compounds in *Ruellia tuberosa* have shown promising potential as drug candidates particularly through their ability to induce intracellular reactive oxygen species (ROS) and trigger apoptosis via mitochondrial pathways. Methanolic extracts from *R. tuberosa* flowers have been reported to promote ROS generation, cause G0/G1 cell cycle arrest, and activate intrinsic apoptosis in cancer cells, while maintaining a favorable safety profile in animal studies. These findings support further exploration of *R. tuberosa* extracts for their immunomodulatory and neuroprotective roles in MS therapy development (10)

This study aims to investigate the metabolites of *Ruellia tuberosa* through a comprehensive *in silico* approach to identify potential drug candidates for multiple sclerosis. The research focuses on computationally evaluating the pharmacological profiles, therapeutic relevance, and predicted mechanisms of action of these compounds, with emphasis on their immunomodulatory and neuroprotective potential. Additionally, toxicity predictions are carried out to assess safety profiles and reduce the risk of adverse effects. This *in silico* exploration is intended to support the early-stage identification of promising, plant-based therapeutic agents for Multiple Sclerosis.

MATERIALS & METHODS

Identification of Phytochemical Compound from *R. tuberosa*

The identification of phytochemical compounds from *R. tuberosa* was performed using the KNApSAcK System (http://www.knapsackfamily.com/). The species name "*Ruellia tuberosa*" was used as a search keyword in the database interface The resulting list of metabolites was retrieved along with compound identifiers (C_ID), CAS numbers, and chemical names. Each selected metabolite was cross-checked in PubChem (https://pubchem.ncbi.nlm.nih.gov/) to ensure structure availability and to retrieve the canonical SMILES format for computational input. The identified bioactive compound would be evaluation with Lipinski's Rule of Five (Ro5).

Drug-Likeness Evaluation Based on Lipinski's Rule of Five

The selected compounds from *R. tuberosa*, obtained through the KNApSAcK Core database, were further evaluated for drug-likeness properties using Lipinski's Rule of Five. Lipinski's Rule of Five is a set of criteria widely used to evaluate the drug-likeness of compounds, particularly their oral bioavailability. According to Lipinski, a compound is more likely to have good absorption and permeation when it meets the following conditions: no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight under 500 daltons, and a calculated log P (octanol-water partition coefficient) not greater than 5. These parameters were

derived from an analysis of compounds that reached Phase II clinical trials, reflecting common physicochemical properties of orally active drugs. The rule serves as a practical guideline in drug discovery to reduce attrition by selecting compounds with favorable pharmacokinetic profiles, although it is not absolute and may not apply to drugs relying on active transport mechanisms (11). This evaluation was conducted using the SwissADME web tool (http://www.swissadme.ch/), with canonical SMILES of each compound retrieved from the PubChem database. Compounds that satisfied at least three of these four rules were considered to have favorable drug-like characteristics. This filtering step served as a preliminary assessment before advancing to toxicity and target activity prediction.

Toxicity Prediction of *R. tuberosa* Bioactive Compound

The compounds that fulfilled Lipinski's Rule of Five were subsequently subjected to toxicity prediction using ProTox-II, an online tool for in silico toxicity profiling (https://tox.charite.de/protox3/). Canonical SMILES obtained from PubChem were used as input to predict the median lethal dose (LD_{50}) in mg/kg body weight, as well as the toxicity class level based on OECD (Organization for Economic Co-operation and Development). The OECD toxicity level classification provides a standardized framework for assessing chemical hazards, particularly through acute toxicity testing, which determines the lethal dose (LD50) values that cause mortality in test animals. Chemicals are categorized into five hazard classes based on their LD50 values via different exposure routes such as oral, dermal, and inhalation, with Category 1 representing the highest toxicity (LD50 ≤ 5 mg/kg orally) and Category 5 indicating the lowest toxicity (LD50 between 2000 and 5000 mg/kg) (12). The compounds that have moderate to high predicted toxicity levels were eliminated at this stage.

Molecular Target Prediction

Compounds that passed the toxicity screening were further analyzed using SwissTargetPrediction (http://www.swisstargetprediction.ch/) to identify their potential biological targets in the human body. This step aimed to predict the most likely macromolecular targets based on the 2D and 3D similarity of each compound to known ligands. Canonical SMILES from PubChem were used as input for each compound. The target profiles were then compared to known therapeutic targets associated with MS to assess the potential of drug candidates.

Prediction of Pharmacological Activities and Corellation with Multiple Sclerosis

Biological activity predictions were conducted using the PASS Online tool (http://www.way2drug.com/passonline) to evaluate the potential pharmacological roles of the selected compounds. The canonical SMILES of each compound were submitted to obtain predictions of probable activity (Pa) across a wide range of known pharmacological functions. Compounds with Pa values greater than 0.7 were considered to have a high likelihood of exhibiting the corresponding biological activities. The predicted activities were then reviewed for their relevance to the multiple sclerosis, particularly those associated with neuroinflammation, immunomodulation, oxidative stress inhibition, or neuroprotective effects.

RESULT AND DISCUSSION

Phytochemical Screening from *R. tuberosa*

The phytochemical profiling of *R. tuberosa* through the KNApSAcK database (Table 1) identified five structurally distinct metabolites: hentriacontane, nonacosane, campesterol, (–)-β-sitosterol, and β-stigmasterol. These compounds represent two major chemical classes, which are long-chain aliphatic hydrocarbons and plant-derived sterols. These phytochemicals are commonly associated with various biological activities.

Tabel 1. Phytochemical Compounds from *Ruellia tuberosa* based on KNApSAcK

C_ID	CAS ID	Metabolite	Molecular formula
C00001250	630-04-6	Hentriacontane	$C_{31}H_{64}$
C00001260	630-03-5	Nonacosane	$C_{29}H_{60}$
C00003647	474-62-4	Campesterol	$C_{28}H_{48}O$
C00003672	83-46-5	(-)-beta-Sitosterol	$C_{29}H_{50}O$
C00003674	83-48-7	beta-Stigmasterol	$C_{29}H_{48}O$

Hentriacontane is a long-chain saturated hydrocarbon that has been reported to exhibit notable bioactive properties, including anti-tumor effects. This compound identified in various plant extracts and demonstrated pharmacological potential even at low concentrations, suggesting its possible contribution to the therapeutic profile of phytomedicinal formulations (13). Hentriancotane found in *R. tuberosa* suggest the phytochemical has a potential for drug candidate. Nonacosane is a straight-chain hydrocarbon commonly found in vegetables and constitutes a major component of plant waxes. Nonacosane only partially absorbed in the gastrointestinal tract due to its high lipophilicity and waxy nature (14). This limited absorption suggests that nonacosane may have low oral bioavailability, which could constrain its potential as a drug candidate. Nonetheless, this hypothesis requires further evaluation through pharmacokinetic predictions, such as those provided by ADME analysis.

Campesterol is an ergostane-type phytosterol commonly derived from plant sources. It has been reported to exhibit diverse physiological functions, including antibacterial, anticancer, and cardioprotective activities. Due to its structural similarity to endogenous steroids, campesterol holds potential as a scaffold for the development of steroid-based therapeutics, and it's possible to be a drug candidate for Multuple sclerosis (15).

Stigmasterol and β-sitosterol are among the most abundant phytosterols, which serve as plant-derived analogues of cholesterol. These compounds have been shown to significantly ameliorate high-fat Western diet (HFWD)-induced hepatic steatosis and associated metabolic disturbances, including elevated levels of total hepatic lipids, triacylglycerols, and cholesterol, as well as pathological alterations in liver tissue. Mechanistically, their effects have been linked to the modulation of gene expression related to lipid metabolism and homeostasis (16). Given their structural similarity to endogenous sterols and their ability to influence metabolic and inflammatory pathways, both stigmasterol and β-sitosterol hold considerable promise as drug candidates. In the context of multiple sclerosis, these phytosterols may contribute to immunometabolic regulation and exert neuroprotective effects by modulating lipid-mediated signaling and reducing neuroinflammation.

Phytochemicals Screening Based on Lipinski's Rule of Five

Drug-likeness evaluation was conducted using Lipinski's Rule of Five (Ro5), which serves as a widely accepted guideline for predicting the oral bioavailability of small molecules. All five bioactive compounds identified from *R. tuberosa* fulfilled at least three out of the four Ro5 parameters, thereby qualifying them as orally acceptable candidates. Ro5 screening of five compounds (Table 2) shows that all compounds exhibited molecular weights below 500 g/mol, zero or one hydrogen bond donors (HBD), and fewer than ten hydrogen bond acceptors (HBA). These values align with standard pharmacokinetic expectations for drug absorption and distribution, supporting their potential for further development.

Table 2. Lipinski's Rule of Five Screening of *R. tuberosa* Phytochemical

Lipinski's Rule of Five (Ro5) Criteria	Max Limit	Bioactive Compounds				
		Hentriacontane	Nonacosane	Campesterol	(-)-beta-Sitosterol	beta-Stigmasterol
Molecular Weight (MW)	≤ 500 g/mol	434.84 g/mol	408.79 g/mol	400.68 g/mol	414.71 g/mol	412.69 g/mol
LogP	≤ 5	11.9	11.18	6.92	7.24	6.98
HBD	≤ 5	0	0	1	1	1
HBA	≤ 10	0	0	1	1	1
Bioavailability	>10%	55%	55%	55%	55%	55%
Lipinski Violation	≤ 1	1	1	1	1	1
Drug Candidate Status		Acceptable	Acceptable	Acceptable	Acceptable	Acceptable

Despite these favorable parameters, all compounds recorded LogP values exceeding the recommended threshold of 5, indicating high lipophilicity. The biggest LogP values are Hentriacontane and Nonacosane, which higher than 11. LogP, which reflects the lipophilicity of a compound, is a critical parameter within Lipinski's Rule of Five for assessing oral drug-likeness. High LogP values, particularly those exceeding 5, are commonly associated with poor aqueous solubility, which can significantly impair gastrointestinal absorption and reduce systemic bioavailability. Moreover, compounds with excessive lipophilicity may present formulation challenges and exhibit a higher risk of nonspecific binding or toxicity (17). Despite of High LogP value, all five phytochemical compounds fulfilled at least three of the Lipinski's Rule of Five criteria, indicating acceptable oral drug-likeness, further evaluation through *in silico* toxicity testing was necessary to assess their safety profiles.

Toxicity Prediction of Phytochemicals

In silico toxicity screening was carried out using ProTox-II to evaluate the safety profiles of the selected phytochemicals. The result (Table 3) shows that Hentriacontane and nonacosane exhibited LD_{50} values of 750 mg/kg and were classified under toxicity class 3, indicating moderate toxicity. This level of toxicity suggests that although these compounds possess acceptable drug-likeness, their therapeutic use may require careful dose regulation or alternative delivery strategies to mitigate potential toxic effects. Their high LogP values observed in the Ro5 evaluation may contribute to their increased toxicity due to reduced solubility and possible accumulation in lipid-rich tissues. These findings suggest that hentriacontane and nonacosane are less suitable as potential drug candidates for multiple sclerosis therapy, primarily due to their moderate predicted toxicity. In the development of therapeutics for chronic neuroinflammatory diseases such as MS, minimizing side effects is a critical consideration, as adverse reactions can exacerbate disease symptoms or compromise patient quality of life. The moderate toxicity levels observed for these two hydrocarbons raise concerns regarding their safety margins, which could limit their applicability or necessitate significant formulation modifications for clinical use.

Table 3. Toxicity Screening of *R. tuberosa* Phytochemical

Bioactive Compound	Predicted LD50	Predicted Toxicity Class	Toxicity Status
Hentriacontane	750 mg/kg	3	Moderately toxic
Nonacosane	750 mg/kg	3	Moderately toxic
Campesterol	890 mg/kg	4	Slightly toxic
(-)-beta-Sitosterol	890 mg/kg	4	Slightly toxic
beta-Stigmasterol	890 mg/kg	4	Slightly toxic

In contrast, campesterol, (-)-β-sitosterol, and β-stigmasterol demonstrated slightly higher LD_{50} values (890 mg/kg) and were categorized under class 4, indicating lower acute toxicity. These findings align with their known safety in various dietary and nutraceutical applications. Their favorable toxicity profiles, combined with acceptable Ro5 compliance and relevant pharmacological potential, support their prioritization as promising candidates for further investigation in the context of neuroimmune-modulating therapies, particularly for multiple sclerosis.

Drug Target Prediction of Phytochemicals

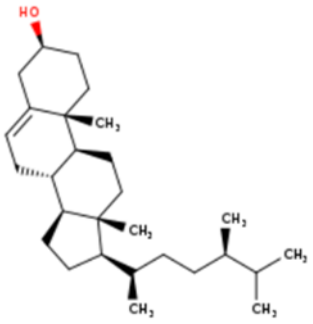
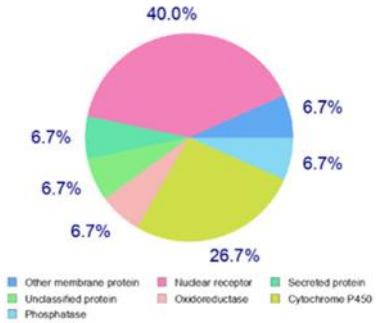
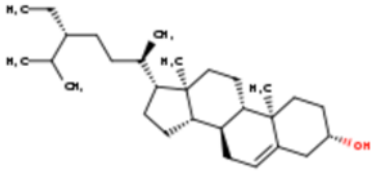
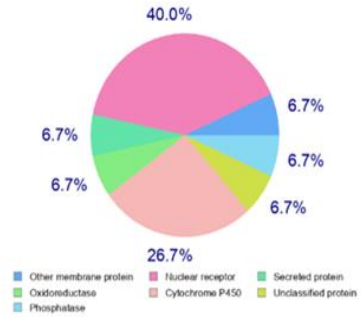
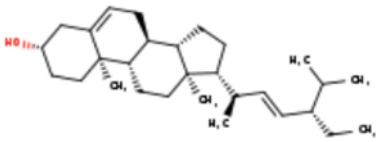
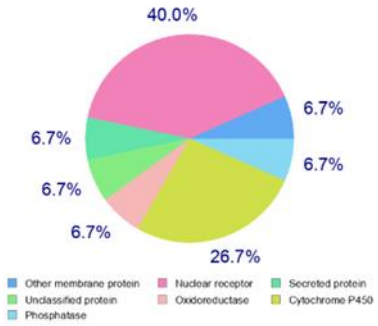
The predicted target profile (Table 4) for campesterol reveals a strong association with nuclear receptors (40%). This result consistent with its sterol-based structure, which resembles endogenous ligands for nuclear hormone receptors. Additionally, campesterol also shows moderate binding potential to cytochrome P450 enzymes (20%) and a diverse array of other classes including phosphatases, oxidoreductases, and membrane proteins. The target prediction for (–)-β-sitosterol similarly highlights a predominant interaction with nuclear receptors (40%), followed by cytochrome P450 enzymes (26.7%). The remaining targets are distributed across membrane proteins, oxidoreductases, and secreted proteins. The substantial predicted binding to cytochrome P450 family members indicates a potential modulatory role in xenobiotic metabolism and steroid biosynthesis. For β-stigmasterol, the target distribution also shows nuclear receptors as the primary predicted class (40%), with unclassified proteins comprising 26.7% of the targets, followed by modest associations with membrane proteins and oxidoreductases. The significant interaction with unclassified proteins suggests additional unexplored molecular pathways that could contribute to its pharmacological effects.

Nuclear receptors emerged as the predominant class of predicted targets across multiple bioactive compounds. These receptors are central regulators of lipid metabolism and inflammatory signaling, both of which are critically implicated in the pathogenesis of multiple sclerosis. Their functional relevance is their involve in modulating immune cell behavior, particularly macrophage polarization and cytokine expression (18). Receptors such as the glucocorticoid and androgen receptors have been shown to influence neuroimmune interactions, suggesting that targeting these pathways may offer therapeutic benefit in controlling disease progression in multiple sclerosis.

Following nuclear receptors, cytochrome P450 (CYP450) enzymes were identified as key predicted targets for three of the evaluated phytochemicals. The CYP450 superfamily plays a critical role in the metabolism of numerous endogenous and exogenous compounds, including several drugs used in the treatment of multiple sclerosis. Notably, enzymes such as CYP4F are involved in the metabolic clearance of fingolimod (FTY720), a first-in-class oral therapy for relapsing forms of multiple sclerosis (19). This underscores the relevance of CYP450 enzymes in shaping the pharmacokinetics, bioavailability, and therapeutic efficacy of Multiple Sclerosis medications. Beyond their metabolic functions, CYP450 enzymes also participate in immunoregulatory pathways relevant to Multiple Sclerosis pathogenesis. For example, polymorphisms in CYP27B1, an enzyme involved in the activation of vitamin D_3, have been associated with altered immune responses and increased susceptibility to multiple sclerosis (20). Since vitamin D plays a well-established role in modulating inflammatory and neuroimmune functions, targeting CYP450 pathways may provide both metabolic and immunological leverage in Multiple Sclerosis therapy. CYP450 as a predicted target supports the potential of the selected phytochemicals to influence not only drug metabolism but also immune regulation. These dual roles highlight the importance of

CYP450-targeting agents in the broader context of MS drug discovery, particularly for the development of compounds with both therapeutic and immunomodulatory benefits.

Table 4. Drug Target Prediction of *R. tuberosa* Phytochemical

Phytochemical Compound	Structure	Target Classes
Campesterol		
(-)-beta-Sitosterol		
beta-Stigmasterol		

Beside nuclear receptors and cytochrome P450 enzymes, a notable proportion of predicted targets were classified as oxidoreductases. These enzymes play a pivotal role in maintaining redox homeostasis and regulating oxidative stress, both of which are fundamental to the pathophysiology of multiple sclerosis. Oxidative stress, characterized by the excessive accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), contributes to mitochondrial dysfunction, lipid peroxidation, and subsequent neuronal injury, hallmark features of MS pathology. Activated astrocytes and macrophages in MS lesions are known to generate high levels of ROS and RNS, exacerbating inflammation and demyelination. In response, several oxidoreductase enzymes, such as NAD(P)H:quinone oxidoreductase 1 (NQO1), superoxide dismutases (SODs), and catalase, are upregulated as part of the endogenous antioxidant defense system. These enzymes act to neutralize oxidative agents, thereby protecting neural tissue and slowing disease progression. The identification of oxidoreductases as molecular targets suggests that the candidate compounds may confer neuroprotection

through redox modulation, offering a complementary mechanism alongside immunoregulation for MS therapy (21).

Targeting oxidoreductases offers a compelling therapeutic approach for mitigating oxidative stress-related damage in multiple sclerosis. These enzymes support redox homeostasis and counteract the accumulation of reactive oxygen and nitrogen species that contribute to neuroinflammation, demyelination, and neuronal loss in MS pathology. Central to this defense is the Nrf2–ARE (antioxidant response element) pathway, which regulates the expression of key oxidoreductases such as NQO1, superoxide dismutases, and catalase. Activation of this pathway has been shown to protect oligodendrocytes and neurons, reduce inflammation, and slow disease progression (22). Moreover, certain oxidoreductase enzymes, such as NAD(P)H:quinone oxidoreductase 1 (NQO1), are markedly upregulated in active demyelinating lesions of multiple sclerosis, reflecting their dual roles in detoxification and antioxidant defense. Pharmacological enhancement of these enzymes has the potential to attenuate oxidative injury while simultaneously modulating immune activity, thereby reducing lesion formation and limiting disease progression. This mechanistic insight reinforces the therapeutic relevance of oxidoreductase-targeting agents as integral components of multi-modal MS treatment strategies aimed at curbing redox imbalance and its pathological consequences in the central nervous system (23). Therefore, compounds predicted to modulate oxidoreductase activity, including those derived from *R. tuberosa*, may exert neuroprotective effects through redox modulation, positioning them as promising multi-target candidates for multiple sclerosis therapy.

Pharmacological Activity Prediction of Phytochemicals

Three selected phytochemical compounds were further analyzed to predict their potential biological activities using PASS (Prediction of Activity Spectra for Substances) Online. PASS is a widely recognized computational tool designed to predict the potential biological activities of chemical compounds based on their molecular structure. By leveraging a comprehensive database of bioactive molecules and utilizing Bayesian statistical modeling, PASS estimates the probability of a compound exhibiting a wide spectrum of pharmacological effects. This approach significantly accelerates early-stage drug discovery by enabling virtual screening and prioritization of compounds with desirable therapeutic profiles, thereby minimizing the reliance on extensive in vitro assays. Its high predictive accuracy across diverse activity classes also supports its use in drug repurposing and mechanism-of-action studies (24). Table 5 summarizes the predicted biological activities of selected phytochemicals, with particular emphasis on those functionally relevant to multiple sclerosis pathophysiology.

Table 5. Predicted Biological Activities of Selected Phytochemicals from *R. tuberosa* Based on PASS Online Analysis (Pa > 0.7)

Type of Targets	Phytochemicals					
	Campesterol		(-)-beta-Sitosterol		beta-Stigmasterol	
	Predicted Activity	PA	Predicted Activity	PA	Predicted Activity	PA
Nuclear receptor	Chemopreventive	0.844	Chemopreventive	0.831	Caspase-3 stimulant	0.863
	Caspase-3 stimulant	0.838	Caspase-3 stimulant	0.806	Chemopreventive	0.827
	Immunosuppressant	0.761	Immunosuppressant	0.762	Immunosuppressant	0.782
					Apoptosis agonist	0.753

Cytochrome P450	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0.940	Testosterone 17β-dehydrogenase (NADP+) inhibitor	0.924	Testosterone 17β-dehydrogenase (NADP+) inhibitor	0.915
	Cholesterol synthesis inhibitor	0.767	UDP-glucuronosyltransferase substrate	0.889	UDP-glucuronosyltransferase substrate	0.805
			Cholesterol synthesis inhibitor	0.778	Cholesterol synthesis inhibitor	0.778
Oxidoreductase	Prostaglandin-E2 9-reductase inhibitor	0.962	Prostaglandin-E2 9-reductase inhibitor	0.959	Oxidoreductase inhibitor	0.933
	Oxidoreductase inhibitor	0.884	Oxidoreductase inhibitor	0.886	Prostaglandin-E2 9-reductase inhibitor	0.913
	HMOX1 expression enhancer	0.764	HMOX1 expression enhancer	0.745	HMOX1 expression enhancer	0.729

Among nuclear receptor-associated activities, all three compounds showed a high probability of being chemopreventive agents and caspase-3 stimulants, suggesting roles in modulating apoptosis and cellular stress response pathways. Notably, β-stigmasterol exhibited the highest Pa for caspase-3 stimulation (0.863), further supported by its unique prediction as an apoptosis agonist (Pa = 0.753). Chemopreventive agents have gained increasing attention in the context of multiple sclerosis therapy due to their capacity to modulate immune function and confer neuroprotection. A notable example is dimethyl fumarate, an FDA-approved oral treatment for relapsing forms of MS, which exerts its therapeutic effects primarily through activation of the Nrf2 signaling pathway. This pathway enhances endogenous antioxidant defenses and mitigates oxidative stress within the central nervous system, thereby reducing neuroinflammation and limiting demyelination (25). These protective mechanisms contribute to improved clinical outcomes in MS patients.

R. tuberosa phytochemical also predicted to have Caspase-3 stimulant effect. Caspase-3 plays a pivotal role in the neuronal apoptosis associated with multiple sclerosis, where its activation contributes to the progression of neurodegeneration. High caspase-3 activity has been linked to increased neuronal loss in MS lesions, making it a critical target for therapeutic intervention. Experimental studies have demonstrated that inhibition of caspase-3 using agents such as Ac-DEVD-cmk can preserve neuronal viability and reduce DNA fragmentation triggered by cerebrospinal fluid derived from MS patients. These findings highlight the therapeutic potential of caspase-3 modulation as a strategy to mitigate neuronal damage and slow disease progression in MS (26). Furthermore, caspase-3 inhibition has demonstrated neuroprotective effects in animal models of multiple sclerosis by attenuating inflammatory cell death pathways and reducing lesion severity. These findings further underscore the therapeutic relevance of targeting caspase-3 in efforts to limit neuroinflammation and promote neuronal survival in MS (27).

One of the phytochemicals identified from *R. tuberosa* was predicted to exhibit immunosuppressive potential, which is highly relevant in the context of multiple sclerosis therapy. Immunosuppressive agents have been employed in MS management since the 1960s, primarily targeting the autoimmune mechanisms that underlie the disease. By inhibiting immune cell proliferation and modulating pro-inflammatory cytokine profiles, immunosuppressants help attenuate demyelination and neuroinflammation, as key pathological features of multiple sclerosis. Their use is particularly valuable

for patients who do not respond adequately to first-line disease-modifying therapies, offering an alternative therapeutic avenue (28). As research continues to optimize immunosuppressive strategies, identifying phytochemicals with such activity could contribute to the development of novel, plant-derived MS treatments.

Under the cytochrome P450 category, phytochemicals derived from *R. tuberosa* was predicted to interact with Testosterone 17β-dehydrogenase (17β-HSD), an enzyme that plays a pivotal role in steroid hormone metabolism. This enzyme catalyzes the bidirectional conversion between active and inactive forms of androgens such as testosterone, which are known to exert neuroprotective and immunomodulatory effects (29). Testosterone itself is known for its anti-inflammatory, neuroprotective, and promyelinating effects in the CNS, mediated through androgen receptors (30). In multiple sclerosis, dysregulated steroidogenesis, has been observed and is thought to contribute to disease pathophysiology. Particularly in female MS patients, impaired 17β-HSD-mediated pathways may lead to reduced levels of circulating testosterone and its derivatives, thereby compromising endogenous neuroprotection and enhancing pro-inflammatory responses (29). The prediction that a *R. tuberosa* compound may modulate this enzyme suggests a potential mechanism through which plant-derived metabolites could influence hormonal regulation and immune modulation in MS.

Campesterol and β-sitosterol also showed potential as cholesterol synthesis inhibitors, while both sitosterol and stigmasterol were predicted to interact with UDP-glucuronosyltransferase substrates. Cholesterol synthesis inhibitors have shown promising immunomodulatory potential in the context of multiple sclerosis. For instance, atorvastatin has been reported to modulate immune function by promoting a shift from pro-inflammatory Th1 responses toward anti-inflammatory Th2 profiles, while also reducing major histocompatibility complex class II expression on antigen-presenting cells and suppressing central nervous system inflammation in experimental autoimmune encephalomyelitis models (31). Based on PASS prediction results, phytochemicals from *R. tuberosa*, including β-sitosterol and stigmasterol, exhibited predicted activity as cholesterol synthesis inhibitors. This suggests that these compounds may exert similar immunomodulatory effects, potentially contributing to a reduction in neuroinflammation and disease severity in multiple sclerosis. Their predicted roles in lipid metabolism and immune regulation provide further support for their candidacy as orally active, plant-derived adjunct therapies for multiple sclerosis treatment.

UDP-glucuronosyltransferases (UGTs) are phase II detoxification enzymes that catalyze the conjugation of glucuronic acid to a wide range of lipophilic substances, enhancing their solubility and elimination from the body. In the context of multiple sclerosis, modulation of UGT activity is relevant due to its role in the metabolism of neuroactive steroids and other inflammatory mediators. Although direct associations between specific UGT substrates and multiple sclerosis pathogenesis remain underexplored, alterations in UGT activity, such as those regulated by protein kinase C-mediated phosphorylation, can affect the homeostasis of compounds implicated in neuroinflammation and neurodegeneration (32). In this study, PASS prediction indicated that several *R. tuberosa* phytochemicals, including campesterol, β-sitosterol, and stigmasterol, are potential substrates of UGT1A and UGT2B families. These findings suggest that their metabolism and biological activity may be influenced by UGT-mediated glucuronidation, potentially modulating their pharmacokinetics and therapeutic efficacy in the treatment of multiple sclerosis.

Within the oxidoreductase category, all three compounds demonstrated strong predicted activity as inhibitors of prostaglandin-E2 9-reductase and general oxidoreductase enzymes, with Pa values exceeding 0.88.

Campesterol showed the highest Pa (0.962) for prostaglandin inhibition, aligning with its anti-inflammatory profile. Additionally, all three compounds were predicted to enhance HMOX1 expression, a key cytoprotective gene involved in redox balance and neuroprotection. Prostaglandin-E2 9-reductase inhibitors, which modulate prostaglandin E2 (PGE2) biosynthesis, hold significant potential in the context of multiple sclerosis due to the central role of PGE2 in orchestrating immune responses and sustaining chronic neuroinflammation. Elevated PGE2 levels are associated with immunosuppressive microenvironments that exacerbate inflammatory cascades and tissue damage in the central nervous system. Selective inhibition of microsomal prostaglandin E synthase-1 (mPGES-1), a key enzyme downstream of cyclooxygenase, has demonstrated the ability to suppress neuroinflammation without interfering with the synthesis of other physiologically important prostaglandins (33). Inhibiting prostaglandin-E2 9-reductase or mPGES-1 may help modulate immune responses, reduce neuroinflammation, and limit relapse severity, making these enzymes attractive therapeutic targets (34). This approach offers a more favorable safety profile compared to traditional COX inhibitors. In the current study, the PASS prediction indicated that several phytochemicals derived from *R. tuberosa* possess potential activity as Prostaglandin-E2 9-reductase inhibitors, suggesting that they may exert anti-inflammatory effects by modulating PGE2 signaling. This mechanism provides further rationale for exploring these compounds as therapeutic candidates in managing inflammation-driven neurodegenerative processes.

Heme oxygenase-1 (HMOX1) provides neuroprotective and anti-inflammatory effects in multiple sclerosis by reducing oxidative stress. Its increased expression in both patient tissues and animal models is associated with milder disease symptoms. Inducing HMOX1 has been shown to suppress inflammation and limit disease progression, partly through the beneficial effects of carbon monoxide, a byproduct that reduces immune cell infiltration in the central nervous system. Reduced HMOX1 expression in peripheral immune cells during multiple sclerosis relapses suggests that low HMOX1 activity may worsen inflammation. Compounds that enhance HMOX1 or release CO have shown beneficial effects in experimental models, supporting their potential as supportive therapies to control immune responses and oxidative damage (35,36). The PASS analysis revealed that certain phytochemicals from *Ruellia tuberosa* may enhance HMOX1 expression, a mechanism known to offer neuroprotective benefits in multiple sclerosis by alleviating oxidative stress and regulating inflammatory responses. This prediction aligns with previous evidence showing that elevated HMOX1 activity correlates with reduced demyelination and improved clinical outcomes. Compared to synthetic HMOX1 inducers such as hemin or cobalt protoporphyrin IX, natural compounds from *R. tuberosa* may provide a safer and more accessible alternative with fewer side effects.

CONCLUSION

This in silico study demonstrates that phytochemical compounds from *Ruellia tuberosa*, particularly campesterol, beta sitosterol, and stigmasterol, exhibit favorable drug likeness, low predicted toxicity, and multi target pharmacological profiles relevant to the pathophysiology of multiple sclerosis. Their predicted interaction with nuclear receptors, cytochrome P450 enzymes, oxidoreductases, and neuroimmune regulatory pathways, including inhibition of cholesterol synthesis, modulation of prostaglandin E2 activity, and enhancement of HMOX1 expression, suggests potential roles in immunomodulation, anti-inflammatory activity, and neuroprotection. These results highlight the therapeutic potential of *Ruellia tuberosa* phytochemicals as safe, plant derived candidates for future development of

supportive treatments targeting inflammation and oxidative stress in multiple sclerosis.

Declaration by Authors

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